XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

VE1 - CHAGAS DISEASE IN MEXICO: MORBIDITY, MORTALITY, RISK AREAS AND DISEASE BURDEN.

Ramsey J.M., Ordóñez R., Tello López A., Pohls J.L., Sánchez-Cordero V., Peterson A.T.

Centro de Investigaciones sobre Enfermedades Infecciosas (CISEI), Instituto Nacional de Salud Pública (INSP), Cuernavaca, Morelos, México, Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F., Natural History Museum, The University of Kansas, Lawrence, Kansas USA

Chagas disease is the most important parasitic disease in Latin America, as in Mexico, where 1.5-2% of blood donations are contaminated with anti-*Trypanosoma cruzi* antibody. Through environmental and population based stratification, we estimate that 91 million inhabitants are at risk (78% through residence), 1,768,376 individuals are infected, and mortality may oscillate between 25,500 and 63,000 individuals/yr (830 of these are under 5 yrs old). The disease incidence is estimated at 69,000 cases/yr and approximately 530,500 individuals are currently in chronic phase. More than 96% of the transmission occurs via the vector, and niche modeling with GARP estimates that 67% of the transmission occurs via one of the 6 primary *phyllosoma* complex species.

The economic loss due to incapacity is estimated at US$ 3,160,000,000/yr, while diagnostic and treatment costs currently could attain US$ 126,000,000/yr. In the absence of a vector control program, disease burden could duplicate in 25 yrs, while chronic case treatment will augment by a factor of 45 over the same period.

VE2 - MORPHOMETRIC ANALYSIS OF MEXICAN AND GUATEMALAN POPULATIONS OF *TRIATOMA DIMIDIATA*.

Ramsey J.M., Lehmann P., Monroy C., Bargues M.D., Ordoñez R.

Center for Infectious Disease Research, National Institute for Public Health, Cuernavaca, Morelos, Mexico

Current estimates suggest that 2% of the Mexican population is seropositive for *Trypanosoma cruzi*, and the incidence of Chagas disease may supercede 69,000 cases/yr, with mortality equally 5-6% (25,000 cases) of the country´s total burden. Among the 18 vector species found in the country, *Triatoma dimidiata* plays a critical role in transmission principally in Gulf coast and all states south of the Istmus of Tehuantepec. Current molecular studies using ITS-2 rDNA and mitochondrial DNA markers, suggest that the populations found in the Yucatan peninsula may be an ancestral stock, from which other Mexican, Mesomerican, and Colombian/Venezuelan populations derived. Given profound genetic differences between yucatecan and all other populations, phenotypic differences might be expected using head and wing morphometric and symmetry discriminate analysis. 187 specimens of *T. dimidiata* collected from the states of Yucatán, Veracruz, and San Luis Potosí (SLP), were compared to a population from the Petén, Guatemala, and *T. phyllosoma* from Oaxaca as outgroup (:33 specimens per species). 12 head and 14 wing characters were measured separately for male and female samples, and measurements analyzed using multigroup principal component and discriminate analyses, sexual dimorphism and wing asymmetry. Guillaumin profiles indicated no size difference between SLP and Veracruz populations, while Peten specimens were smaller, and those from the Yucatan the smallest. None of the populations were separated completely using principal component analysis, although there was a clear tendency for separation. No shape differences were observed between SLP and Veracruz populations although they were clearly separated from both the Yucatan and Peten populations using discriminate analysis. Yucatan and Peten populations showed a clear tendency for separation, although this was not complete. Sexual dimorphism and fluctuating wing asymmetry were observed within all populations. There is a substitution of 24 to 27 nucleotides in the ITS-2, and a sequence divergence using Cyt*b* and ND4 mtDNA markers from 3.9 –14.1% between Yucatan and all other populations studied herein. While SLP and Veracruz populations appear to be identical morphometrically, they were clearly differentiated from the other two populations. This was not the case between the Peten and Yucatan populations. Genetic distance between the latter two populations was not reflected in differences in head and wing phenotype characters. Future studies with other marker systems may elucidate this disparity. From an operational viewpoint, head characters were the most informative to differentiate among all populations, suggesting their potential use for differentiating re-infestations during control programs.

VE3 - EGGSHELLS MORPHOMETRY AND MORPHOLOGY OF *TRIATOMA COSTALIMAI, TRIATOMA GUAZU* AND *TRIATOMA WILLIAMI* (HEMIPTERA, REDUVIIDAE).

Oliveira, L G B de; Rosa J A da

Departamento de Ciências Biológicas – Faculdade de Ciências Farmacêuticas – UNESP – Rodovia Araraquara – Jaú Km 1, CEP 14801-902 - Araraquara-SP, Brasil. E-mail: gustavobaffi@yahoo.com.br; rosaja@fcfar.unesp.br

The morphometrics and morphologic researchs contribute for the specific characterization as well as for the phylogenetics studies of the Triatominae. Then was performed a morphometric and morphologic study of *T. costalimai, T. guazu* and *T. williami.* With regard to morphometry of the eggshell of *T. guazu* the averages had been, length of 2.24mm, width of 1.44mm and diameter of opercular opening of 0.59mm. *T. costalimai* presented the following average measurement: length of 2.14mm, width of 1.31mm and diameter of 0.53mm. *T. williami* got a average in the measurement of 2.04mm of length, 1.16mm of width and 0.60mm of diameter. The formats of the three species had presented ellipsoid eggshell and the lateral flattening was present in the three species also. The presence of neck and of collar it was not verified in none of the three species, it was verified the presence of a band in very narrow ring form contiguous and plain that makes the connection between operculum and the body of eggshells. The examination by means of scanning electron microscope showed that the exochorio of the body of *T. costalimai* presented exochorial cells with pentagonal and hexagonal formats, the junctions are little evident, the cells has scasse and unclear perfurations. *T. guazu* presented plain exochorial cells with no symmetrical form (pentagonal and hexagonal) the junction between the cells is well evident, the perforations are concentrate in the internal face of the exochorial cells and the number of perforations varied of 16 the 20 for cell. *T. williami* presented a predominance of hexagonal exochorial cells that was bigger than in *T. guazu* and *T. costalimai,* the junction between the cells of the exochorio is well evident, the cellular exochorio perforations is distributed in irregular way (center, edge and junction) and the number of perforations exceeded 20 for cell. About relation aeropyle and micropyle these structures had been evidenced in the three studied species. Amongst three studied species *T. guazu* presented the biggest length (2.16mm) and the biggest width (1.39mm). *T. williami* showed the average greater in the diameter of the opening (0.60mm). Morphologically, *T. costalimai, T. guazu* and *T. williami* presented lateral flattening but none of them have neck e collar. *T. costalimai* presented the exochorial cells with well distinct characteristics of the others two species (edges little defined, little or inexistence of perforations, plain surface). *T. williami* presented exochorial cells with size and number of bigger perforations than the others two studied species.

This reseach is sponsored by: Fapesp- Processes 01/11081-4 and 1997/10708-6.

VE4 - EVALUATION OF VETORIAL CAPACITY OF *TRIATOMA INFESTANS* AND *TRIATOMA KLUGI* HYBRIDS

Martins, J.F.O.W, Zimmermann, N.,Rona L.D., Carvalho-Pinto C.J.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Caixa postal 476, Florianópolis, SC, Brazil, 88040-900. E-mail: ccb1ccp@ccb.ufsc.br

The genus *Triatoma* is wide spread in Latin American Countries, comprising sylvatic, peridomestic and domestic triatomines, which represents almost 50% of all triatomine species. Some species of the genus *Triatoma* such as *T. infestans* have great importance in the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease. Our group has recently described a new silvatic triatomine species (*Triatoma klugi*) in the State of Rio Grande do Sul (Southern Brazil). Having successfully established a colony of *T. klugi*, we have detected the ability of these insects to mate with *T. infestans* under laboratory conditions. Previous data showed that couples formed by *T. klugi* females and *T. infestans* males produce of high number of viable eggs. The aim of this work was to evaluate the vectorial capacity of *T. infestans*/*T. klugi* hybrids. For that, groups of 50 4th-5th instars of hybrids (*T. klugi* females/*T. infestans* males) were fed for 2-3 hours on anesthetized Swiss mice in the peak of blood parasitemia, infected with *T. cruzi* (Y strain) and *T. rangeli* (Choachi strain). Nymphs of *T. infestans* and *T. klugi* were infected with the same strains and used as control. Triatomines were searched for the presence of flagellates in their feces and hemolymph every 30 days. The infection rate of hybrids was of 70.6% for *T. cruzi* and no *T. rangeli* was observed. The infection rates in control *T. klugi* and *T. infestans* groups was of80.0% and 92% for *T. cruzi* and 42.3% and 30,3% for *T. rangeli*, respectively. Since *T. infestans* and *T. klugi* are sympatric at Rio Grande do Sul State, the high hybrid production and their susceptibility to T. cruzi may assume a epidemiological relevance in the context of Chagas disease.

Supported by UFSC

VE5 - *TRIATOMA INFESTANS* WILD FOCI IN MESOTHERMIC ANDEAN VALLEYS OF COCHABAMBA, BOLIVIA. IMPLICATION OF *TRYPANOSOMA CRUZI* IN NATURAL CYCLE.

CORTEZ M.G.R. *, SOLANO M. **, PINHO A.P. ***, CUERVO P. ****,GRIPP E.C.***, LOROSA E. *,CUPOLILLO, E. *****,FERNANDES O. ****,TORRICO F. **, JANSEN A.M. ***,NOIREAU F. */ ******

*Depto Entomologia, IOC, FIOCRUZ, Rio de Janeiro; **LABIMED, Facultad de Medicina, UMSS, Cochabamba, Bolívia; ***Depto Protozoologia, IOC, FIOCRUZ, Rio de Janeiro; ****Depto Medicina Tropical, IOC, FIOCRUZ, Rio de Janeiro; *****Depto Bioquímica e Biologia Molecular, IOC, FIOCRUZ, Rio de Janeiro; ******IRD, UR016, Montpellier, France

The success of *Triatoma infestans* elimination in Southern Cone countries relied on the fact that this species is almost exclusively domestic. This is not the case of Bolivia where the recent detection of *T. infestans* wild foci throughout various ecosystems calls attention. True wild populations of this species are now well documented from mesothermic Andean valleys (altitude 2,500 m), from the high-Chaco (1,350 m) and the low-Chaco (500 m). With the exception of the arboreal *T. infestans* dark morph from the low-Chaco, all other populations occur in terrestrial habitat.

A survey of *T. infestans* in the wild environment was performed in the Andean focus of Quillacollo, Cochabamba Department. Of 346 traps placed among rocks (30 traps/day), 46% were positive for *T. infestans* counting 478 insects between nymphal instars and adults. Nymphs predominated thoughout and 60% of fecal samples from the examined insects (n =202) were infected with *Trypanosoma sp*. Precipitin test demonstrated that wild *T. infestans* were mostly associated with rodents and marsupials.

Also small rodents (Caviidae and Muridae families) (n = 13) and marsupials (Didelphidae) (n = 3) were examined. Infection by *T. cruzi*, was evidenced in three rodents (23%) and one marsupial (33,33%) by microhematocrit method.

MLEE analysis of 14 genetic loci, demonstrated that all 35 *Trypanosoma cruzi* from wild

T. infestans and four isolates from wild mammals displayed only micro-heterogeneity. PCR amplification of the non-transcribed spacer of the miniexon gene showed that all isolated were in the genotype *T. cruzi* I.

In spite of both genotypes (*T. cruzi* I and *T. cruzi* II) being prevalent in Bolivia, in our study area only *T. cruzi* I is being transmitted wild *T. infestans*, rodents and marsupials.

Supported by CAPES/IRD

VE6 - POPULATION PHENOTYPIC PLASTICITY LINKED TO ECOLOGICAL ADAPTATIONS IN TRIATOMINAE

Abad-Franch F.[1,2], Monteiro F.A.[3], Patterson J.S.[2], Aguilar H.M.[5,6], Beard C.B.[4], Miles M.A.[2]

1-CPqL&MD/FIOCRUZ-AM, Manaus, Brazil; 2-PMBU-ITD, LSHTM, London, UK; 3-IOC/FIOCRUZ-RJ, Rio de Janeiro, Brazil; 4-CDC, Fort Collins, USA; 5-INHMT, Quito, Ecuador; 6-IJCG, Quito, Ecuador.

Triatomine bugs are vectors of *Trypanosoma cruzi* – causative agent of Chagas disease. Sylvatic bug populations maintain enzootic infection cycles by sharing microhabitats with their mammalian hosts; populations adapted to synanthropic environments transmit human Chagas disease. Phenotypic plasticity has been recognised by morphometric and genetic analyses, with examples of convergence and divergence described at various taxonomic levels. Such phenotypic changes have been attributed to ecological transitions: habitat shifts (eg. sylvatic?domestic) involving subsets of genetically homogeneous populations (leading to within-species divergence); or the sharing of comparable habitats by genetically distinct populations (resulting in convergence or the retention of plesiomorphic phenotypes). When using phenotypic characters only, systematists are thus at peril of describing spurious morphospecies or overlooking cryptic taxa.

Following this rationale, we combined phenotypic characterisation (qualitative+quantitative) with mtDNA analysis to study five putative *Rhodnius ecuadoriensis* populations spanning most of the geographic/ecological range of the species (Ecuador: Andean-sylvatic [wet forest], Coastal-sylvatic [seasonally dry forest], Andean-domestic [seasonally humid forest], Andean-domestic [dry forest]; and Peru*: Andean-domestic [very dry forest]).

Qualitative phenotypic assessment revealed differences customarily associated with distinct morphospecies: all synanthropic bugs (3 populations) had comparable, typical phenotypes (small-pale bugs, short-stout heads), whereas Andean-sylvatic specimens were very large and dark with elongated-slender heads. Coastal-sylvatic bugs had intermediate (medium-pale with slender heads) phenotypes. ANOVA and traditional canonical variate analysis (CVA) of head measurements revealed size-related changes associated with microhabitat (large bugs?palms; small bugs?houses). CVA-based reallocation of specimens to their original ecological groups (sylvatic/domestic) was almost perfect (kappa>0.9), supporting the use of discriminant analysis for reinfestation surveillance. While all synanthropic phenotypes were qualitatively comparable regardless of general ecological conditions, sylvatic phenotypes consistently varied when Andean (wet) and coastal (seasonally dry) life zones were compared.

These phenotypic-ecological groups were not recognised by mtDNA analysis. A 663bp fragment of the cytochrome *b* gene was sequenced and analysed using character state- and distance-based methods. Domestic Peruvian bugs (synanthropic-like phenotypes) were separated from the closest Ecuadorian population (coastal-sylvatic) by >3.9% sequence divergence (26 point mutations), suggesting they constitute independent lineages. The maximum distance between Ecuadorian haplotypes was <2% (13 mutations). Andean-sylvatic bugs presented a single haplotype; it was shared with other Ecuadorian bugs (coastal-sylvatic) in spite of their strongly divergent phenotypes. Size-free CVA (head

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

measurement-based) and geometric analysis (wing landmark-based) revealed patterns of difference/similarity compatible with mtDNA-based clades, with a distinct Peruvian cluster. Explicit size partitioning or geometric analysis of form were therefore required for consistently assessing genetic differences using metric data.

These results revealed (1)phenotypic convergence (involving size-related characters) of genetically distinct synanthropic populations (Peruvian?Ecuadorian) and (2)phenotypic divergence within genetically homogeneous clades (sylvatic?synanthropic; coastal-sylvatic?Andean-sylvatic). This remarkable phenotypic plasticity within a single triatomine species was apparently associated with ecological adaptations and microhabitat.

Support: WHO-TDR (A20441, 970195), ECLAT, CNPq. *Provided by Prof. CA Cuba Cuba (Universidade de Brasília)

VE7 - SALIVARY PAF-ACETYLHYDROLASE ACTIVITY OF *TRIATOMA INFESTANS*

Assumpção, T.C.F.; Lozzi, S.P.; Teixeira, A.R.L.; Santana, J.M.

Laboratório Multidisciplinar de Pesquisa em Doença de Chagas, Faculdade de Medicina, Universidade de Brasília, Brasília-DF; corresponding author: jsantana@unb.br.

The saliva of blood-sucking insects shows several pharmacological activities that antagonize the host's hemostatic response. Amongst these activities, the inhibition of platelet aggregation plays an important role as an anti-hemostatic mechanism during blood feeding. In accordance with this feature, we wanted to see if the saliva of *Triatoma infestans* would display hydrolytic activity on the platelet-activating factor (PAF). In this study, a PAF-acetylhydrolase (PAF-AH) activity was identified in the saliva of this insect using the PAF-AH fluorogenic substrate 2-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino) hexanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocoline in a Ca^{+2}-independent manner. The purification of a protein that mediates this enzymatic activity was achieved by a two-step FPLC procedure using ion exchange and hydrophobic interaction columns. The PAF-AH activity was associated with a single 17 kDa saliva protein on SDS-PAGE under reducing conditions. By means of peptide mass fingerprinting analysis it was possible to confirm the identity of the protein as a member of the phospholipase A_2 family. This enzyme was shown to be immunogenic as it was capable to induce specific IgG antibodies in mice. Host PAF hydrolysis by this *T. infestans* enzyme could be related to the inhibition of platelet aggregation, thus helping the insect to obtain his blood meal. Also, it may reduce the inflammation process at the site of the insect bite and that could facilitate the transmission of *Trypanosoma cruzi* to mammal hosts. Furthermore, other possible functions of this activity would be lysis of blood cells and decreasing host's nociceptive response. These features suggest that enzymes with such activity would be good candidates to the development of vaccines against vector-borne diseases like Chagas' disease.

This research is sponsored by: CNPq.

VE8 - PHOSPHOLIPASE INHIBITION PROTECT THE *VENEZA ZONATA* (HEMIPTERA COREIDAE) AGAINST SEPTICEMIA CAUSED BY TRYPANOSOMATID PARASITE 563DT ISOLATED FROM *EUSCHISTUS HEROS* (HEMIPTERA PENTATOMIDAE

Souza, T. A. C. B. [1]; Oliveira, D. [1]; Jankevicius, J. V. [2]; Gaziri, L. C. J. [1] & Itow-Jankevicius, S.[1]

[1] Universidade Estadual de Londrina, Londrina, 86051970, PR, Brasil; [2] Universidade do Norte do Paraná, Londrina, PR, Brasil.* E-mail: tatilaka@bol.com.br

The *Veneza zonata* is a Hemiptera insect of the Coreidae family, whose geographic distribution is wide, ranging from the U.S.A. to South America. These insects feed on corn, sorgo, bean, tomato, soy, guandu and various legumes and fruits. In the Londrina region, the presence of the insect in cornfields is predominant and it is considered a plague by many agriculturists. Besides its agricultural importance, *V. zonata* is frequently infected with trypanosomatids of the *Phytomonas*, *Leptomonas*, *Herpetomonas* and *Crithidia* genera. The 563DT (*Leptomonas*) strain, isolated from the digestive tract of *Euschistus heros* (Hemiptera Pentatomidae), pathogenic for *Veneza zonata*, was analyzed for phospholipases inhibtion in living cells. *Veneza zonata* specimens were collected on rural properties in Londrina, Paraná, southern Brazil. After inoculating *V. zonata* with 563DT strain, insect death was observed in approximately 24 h after infection, with intense bacterial proliferation in the hemocoel. When 563DT trypanosomatids were previously incubated with a phospholipase inhibitor called Palmitoyl-carnitina, there was a relevant reduction in insect deaths, showing that phospholipases are probably involved in the pathogenic mechanism. In a previous work, we showed an increase of survival rate of insect treated with proteases inhibitors. In this work, we observed that insects inoculated with proteases plus phospholipases inhibitors had their survival rate increased. The insects treated with serino-proteases and phospholipases inhibitors presented a survival rate of 91% from control insects inoculated only with sterile NaCl 0.85%.

Key Words: Trypanosomatids, phospholipases, *Veneza zonata*,

VE9 - ISOLATION AND CHARACTERIZATION OF DEFENSIN, AN ANTIMICROBIAL PEPTIDE OF THE CHAGAS DISEASE VECTOR, *TRIATOMA BRASILIENSIS*.

Araujo, C.A.C., [1,2] Waniek, P.J., [2] Jansen, A.M., [1] Kollien, A.H. [2] and Schaub, G.A.[2]

[1]. Department of Protozoology, Institute Oswaldo Cruz, Rio de Janeiro, Brazil. [2]. Department of Special Zoology, Ruhr-University, Bochum, Germany

The humoral immune response in insects includes the synthesis of several classes of proteins, e.g., lysozyme and defensin. However, not only in the haemolymph but also in the gut these antimicrobial peptides appear: cecropin is expressed in the gut of *Drosophila* and *Bombyx* and defensin in the gut of the blood-sucking insects *Stomoxys calcitrans*, *Aedes aegypti*, *Anopheles gambiae* and *Rhodnius prolixus*.[1, 2] Since triatomines ingest sterile blood, there seems to be no necessity for intestinal antibacterial compounds. However, triatomines swallow air before moulting, offering air-borne bacteria access to the intestine. In addition, the development of triatomines strongly depends on possessing endosymbiotic bacteria, which they obtain via coprophagy. These bacteria multiply after blood ingestion in the cardia and stomach. The passage of the blood from the stomach to the digesting small intestine causes considerable destruction of symbiont populations, and only about 0,01% of the total population is still present in the rectum.[3] Since this development can not be correlated to the activity of lysozyme we are investigating other antibacterial compounds, e.g., defensins.

We have isolated and characterized from the intestine of *T. brasiliensis* the cDNA encoding a defensin gene. The complete nucleotide sequence of 282 bp was amplified by PCR using degenerated oligonucleotides derived from the known amino acid sequences of defensins A, B and C from *Rhodnius prolixus*. RACE was used to amplify the 5'- and 3'-end of the defensin encoding cDNA. The overall amino acid identity between *T. brasiliensis* and *R. prolixus* defensin was ca. 75%. The deduced protein has a size of ca.10 kDa with a putative signal peptide after the amino acid residue *Ser*-19 and an activation cleavage site at *Lys*-50.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Volkswagen Stiftung and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

[1] Munks R.J.L., Hamilton J.V., Lehane S.M., Lehane M.J. 2001. Insect Mol. Biol. 10, 561-571.
[2] Lopez L., Morales G., Ursic R., Wolff M., Lowenberger C. 2003. Insect Biochem. Mol. Biol. 33, 439-447.
[3] Eichler S., Schaub G.A. 2002. Exp. Parasitol. 100, 17-21.

VE10 - EVALUATION OF ITS2 POLYMORPHISM IN *TRIATOMA* SPECIES BY PCR-RFLP

Justino HHG1; Rosa JA da1; Costa PI da2.

[1]Faculdade de Ciências Farmacêuticas, Departamento de Ciências Biológicas, Universidade Estadual Paulista, Rodovia Araraquara-Jaú Km 01, Araraquara 14801-902, SP, Brasil; [2]Faculdade de Ciências Farmacêuticas, Departamento de Análises Clínicas, Universidade Estadual Paulista, Rua Expedicionários do Brasil 1621, Araraquara 14801-360, SP, Brasil.

The internally transcribed spacer 2 (ITS2) of the ribosomal DNA (rDNA) of four *Triatoma* species; *Triatoma arthurneivai*, *T. sordida, T. infestans* and *T. tibiamaculata* was evaluated by restriction fragment lenght polymorphism (RFLP).

T. infestans is considered to be the main vector of Chagas disease in the South America countries, showing a closely association with the man. *T. sordida* is also recognized as an important domestic transmitter. Although *T. arthurneivai* and *T. tibiamaculata* remain in sylvatic ecotypes as secondary vectors, they are competent *T. cruzi* hosts being able to invade houses and to attack man.

T. tibiamaculata previously considered member of the *T. infestans* complex, has its status questioned in accordance with recent mitochondrial DNA (mtDNA) study, showing a tendency to cluster in the vicinity of the *Panstrongylus* clade. The same study includes *T. arthurneivai* within *T. sordida* complex.

In contrast to *T. infestans* and *T. sordida*, *T. arthurneivai* and *T. tibiamaculata* do not have its ITS2 studied.

ITS2 has been shown to be a excellent marker for sistematic and phylogenetic inferences: it accumulated a very substantial degree of structural diversity during evolution, possess great number of copies per cell and display specie homogeneity.

The DNA was extracted from six macerated legs of each live specimen using a protocol of an phenol-cloroform extraction procedure. DNA quantification and purity analysis was performed by spectrophotometry.

ITS2 amplification was carried out by Heminested-PCR. The first reaction was performed in higher hybridization stringency using the primers reported by Bachellerie and Qu: forward 5' GTGAACCTGCGGAAGGATCA and reverse 5' ATCCTGGTTAGTTTCTTTTCCT. The second series was carried out with primers: forward 5' GTCGATGAAGAACGCAG and reverse5' ATCCTGGTT AGTTTCTTTTCCT. For both reactions the amplification parameters and conditions were standardized.

The products obtained of approximately 400 bp were tested against a set of restriction enzimes: AccI, EcoRV, HaeIII, HhaI, HinfI, RsaI, XbaI. The digestion profile were resolving in polyacrylamide gel (10%) visualized by silver staining.

HinfI restriction patterns was the same as waited for *T. arthurneivai* and *T. sordida.* Moreover, *T. tibiamaculata* showed identical profile (4 bands). Although *T. infestans* showed distinct restriction sites (only 3 bands). After restriction with HaeIII and HhaI *T. arthurneivai* and *T. infestans* coincided in its profile (3 and 2 bands respectively) leading a not expected result and displaing a possible ITS2 relationship between these species. *T. infestans* ITS2 still presents restriction sites for RsaI and HhaI, which is not seen in other studied species. Other tested enzimes had not cut the sequence.

These preliminary findings show that ITS2 PCR-RFLP method could be a new and useful tool for sistematic and phylogenetic studies being relatively less laborious and expensive than the sequencing.

This research was sponsored by CAPES

VE11 - LYSOZYMES OF *TRIATOMA INFESTANS*

Kollien, A.H., Gerlach, N., Fechner, S., Waniek, P.J., Schaub, G.A.

Department of Special Zoology, Ruhr-University, Bochum, Germany

Lysozymes act in many animals as part of the non-specific immune response against bacteria. In some animals, which ingest large amounts of bacteria, lysozymes have been adapted to digestive purposes and are produced in the intestine and salivary glands, e.g.*Drosophila melanogaster* or *Anopheles gambiae*. Since triatomines ingest sterile blood, there seems to be no necessity for intestinal lysozymes or other antibacterial compounds. However, like all insects, triatomines swallow air before molting, offering air-borne bacteria access to the intestine. Triatomines also possess symbionts, which they obtain from the faeces of other bugs, i.e. via coprophagy. The symbionts strongly multiply after blood ingestion, mainly in the two anterior midgut regions cardia and stomach. The passage to the digesting and resorptive small intestine causes a considerable breakdown of the symbiont population, and only about 0.01% of the total population is present in the rectum.[1] This development can not be correlated to the lysozyme activity which increases parallel to the number of symbionts in the stomach and is much lower in the small intestine.[2]

Using fifth instars of *Triatoma infestans*, the pH-optimum of antibacterial activity was determined by the lysis of *Micrococcus luteus* cell walls in substrate plate tests. Using haemolymph and homogenates of fatbody, cardia, stomach, three parts of the small intestine, rectum and salivary glands, the activity of all samples showed an optimum at an acidic pH, while only the big, transparent salivary gland D1 and the little yellow salivary gland D2 possessed a second optimum at a basic pH.

After using degenerate oligodeoxyribonucleotide primers and obtaining a 174 bp fragment by PCR-amplification, a cDNA gut library of *T. infestans* was screened with this fragment. A clone containing the 3´-end was isolated. The 5´-end was amplified via RACE. Sequencing of the complete lysozyme cDNA revealed a deduced 417 amino acid sequence with high identity (40-50%) with other chicken-type lysozymes. The expression pattern of the lysozyme gene in the digestive tract of the bugs at different molting and feeding stages showed that this gene was upregulated directly after the molt and after feeding.[3] Investigating the expression of lysozymes in all parts used for activity tests via PCR amplification with specific lysozyme-primers, we obtained DNA-fragments by using cDNA of cardia, stomach, the final part of the small intestine and the salivary glands D2 and D3. Sequencing indicated that different lysozymes are expressed in the gut and salivary glands.

References

[1] Eichler, S., Schaub, G.A. 2002. Exp. Parasitol. 100, 17-21
[2] Ribeiro, J.M.C., Pereira, M.E.A. 1983. Insect. Biochem.14, 103-108
[3] Kollien, A.H., Fechner, S., Waniek, P.J., Schaub, G.A. 2003. Arch. Insect Biochem. Physiol. 53, 134-145

VE12 - USE OF MOLECULAR MARKERS TO STUDY GENETIC VARIABILITY IN THREE POPULATIONS OF *TRIATOMA INFESTANS.*

Gustavo Saldaña [1], Victoria Garcia [2], Patricia Diez [3], Gabriela Murua [3], Mario Baigorin [3] and Rolando Rivera-Pomar [1].

1. Max Planck Institute of Biophysical Chemistry. Goettingen, Germany. 2. Departament of Ecology and Environmental Science, University of Umea, Umea,

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Sweden. 3.PROIMI. San Miguel de Tucuman, UNT. Argentina

The Triatominae is a subfamily of the Reduviidae that is composed of 14 genera and 118 known species. All of them are obligated bloodsuckers, regardless of age and sex. More than half of them were proven to have the ability to carry *Trypanosoma cruzi*, the flagellate that causes Chagas disease. The most important vectors of Chagas disease are *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma dimidiata*, *Triatoma sordida*, *Rhodnius prolixus* and *Panstrongylus megistus* (Schofield 1994). They are distributed from eastern and southern Brasil and from the southern half of Bolivia, down to the Argentinian province of Chubut. They are also present in Paraguay and in the largest part of Uruguay. At the east of the Andes, they can be found in northern Chile and Southern Peru. The species are almost exclusively domestic and peridomestic. Sylvatic colonies are only reported from Bolivia.

In the past decade there has been a remarkable increase in the use of genetic markers to characterize genetic diversity in different species. Some of these genetic markers have a different molecular basis, but all of them are focused to understand the organization of genetics structure of natural and cultivated populations. In additions, these markers have been used to determine the genetics similarity among and within populations avoiding environmental influence.

In this work we show the results of a genetic diversity study on intra an inter-population of *Triatoma infestans*, collected from three different cities in the Argentinean provinces of Cordoba, Catamarca and Mendoza. To obtain fingerprints of each populations a total of 38 RAPD primers belonging to the OPA, OPI and OPB series were assayed. Bands were recorded in the binary form i.e (1)=presence and (0)=absence, and assembled in a data matrix table. The UPGMA algorithm was used for hierarchical cluster analysis. Pairwise comparisons were calculated using Simple Matching (SM) coefficient. In addition a dendrogram was built using NTSYS-pc package (Rohlf 1990).

VE13 - FEEDING BEHAVIOUR OF *TRIATOMA BRASILIENSIS*: INTRAVITAL MICROSCOPY ON HAIRLESS MICE

Guarneri, AA[1], Diotaiuti, L[1], Pereira, MH[2].

[1]Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002, Belo Horizonte, MG, Brasil. [2]Depto. de Parasitologia, ICB, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901, Belo Horizonte, MG.

The feeding behaviour of *T. brasiliensis* on mice was analysed through intravital microscopy. Second (n=19) and third instars nymphs (n=10) were fed on the ear of hairless mice previously anaesthetised. The mouse ear was gently extended over a transparent platform and the animal was then placed under an optical microscope. Microscopy images were recorded by a charge-coupled device video camera and transferred to a video system for off-line analysis. The mean number of bites was 2.6±0.3 bites per insect. The analysis of the images showed that the probing time depends on the proximity between the bite and vessel. Concerning the type of vessels used for feed, 74% of the nymphs fed on venules, 12% on arterioles and 15% on vessels that could not be identified. It was not observed vasodilatation in any of the assays. However, in 43% of the cases it was observed vasoconstriction in the vessel where the mouthparts were inserted. In 62% of the assays where vasoconstriction occurred, this phenomenon was also observed in a vessel near to that in which the insect was feeding. Haemorrhage was observed in 48% of the assays, mainly during the probing time or after the withdrawal of the mouthparts. Interruptions during the bloodmeal were observed in 25% of the experiments. Usually, when interrupted, the triatomine searched for another vessel. The experiments performed through intravital microscopy add information to the study of triatomine feeding behaviour allowing a broad vision of the phenomena that occur during the bloodmeal in mammal hosts.

Financial Support: CNPq, CPqRR/FIOCRUZ, FAPEMIG

VE14 - SYNANTHROPIC TENDENCY OF TRIATOMINAE VECTORS OF TRYPANOSOMATIDAE IN NORTH WESTERN PERU: VECTOR CAPACITY OF *RHODNIUS ECUADORIENSIS, TRIATOMA CARRIONI, PANSTRONGYLUS CHINAI AND PANSTRONGYLUS RUFOTUBERCULATUS*

Cuba Cuba, C.A.

Universidade de Brasilia, Faculdade de Medicina, Área de Patología & Núcleo de Medicina Tropical, Brasilia, DF. Brasil. CEP 70910-900 cesarcuba@hotmail.com /cuba@unb.br

The development of strategies for the adequate control of the vector transmission of Chagas Disease depends upon the availability of updated data on the species of triatomines present in each region, their geographical distribution, natural infection by *Trypanossoma cruzi*, eco-biological characteristics and, synanthropic behavioral tendencies. This paper summarizes and updates, critically, information available in published reports and obtained by our field and laboratory studies over the last three years in the North-Western region of Peru. Resulting from these observations is the realization that three triatomine species exhibit a strong synanthropic behavior and vector capacity, being present into domestic and peridomestic environments: *Rhodnius ecuadoriensis*, *Panstrongylus herreri* (synonymus of *Panstrongylus lignarius*) and *Triatoma carrioni*. The first is the only *Trypanosoma rangeli* corroborated vector, but with sporadic natural infection by *T. cruzi* a situation that apparently has not changed in the last two decades. *P. herreri*, with populations in active geographical expansion, continues to be the most effective vector for *T. cruzi* and of human Chagas Disease in the North-Western region. Currently, *Triatoma carrioni* spreads itself out very quickly in the domiciles of the Sullana and Ayabaca Provinces of Piura Department. The existing range of dispersion toward the south of the country and natural infection by *T. cruzi* are unknown. The three species should be given continual attention by Peruvian public health authorities. The possibility of a favorable vector control strategy would be guaranteed if all of them did not have silvatic populations within the ecosystems in which they are distributed. *Panstrongylus chinai* and *Panstrongylus rufotuberculatus* are bugs with an increasing potential in their role as vectors according to their demonstrated synanthropic tendency, wide distribution, broad ecological valence and, trophy eclecticism. Remains to be proved the real epidemiological role of *Panstrongylus geniculatus*. We do not have explanation yet for the apparent absence of *Triatoma dimidiata* from the previously reported geographic distribution in Peru. There is a pressing need to carry out studies on the genus *Rhodnius* species,to evaluate their present Trypanosomatidae vector capacity in the Peruvian North-Eastern Amazon.

Supported by CNPq, CAPES and British Council, Brazil..

VE15 - THE SERIOUS PROBLEM OF BOLIVIA: CHAGAS DISEASES AND THE BUGS: *TRIATOMA INFESTANS*

Nora Medrano Mercado Msc. PhD (1), Omar, Tenorio Vasquez(1), Merubia Martinez, Marcelo(1), Torrez Tarqui Alejandra(1), Herrera Mesa, René(2), Inturias Alyson(1)

(1)Inmunoparasitology and Chagas'disease Laboratory, Biology Department, San Simón Univeristy, Cochabamba-Bolivia, (2)Chagas'disease Departmental Program Cochabamba-Bolivia

Chagas disease is one of the most serious public health problems in Bolivia, in terms of its magnitude and its social impact. The regions at risk represent 60% of the Bolivian territory and cover more than a half of the municipalities(André Medici,et.al2001). Recall that in 1998, the population at risk was estimated to be more than 4 million people, which would require to

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

spray about 700 thousand households, near 40% of the national total. The population, the number of households and localities in areas at risk of Chagas.Up to three and a half million Bolivians are at risk or already infected with *T. cruzi*, the protozoan parasite that causes Chagas disease. Because treatment options are limited and no vaccine exists, vector extermination and elimination of vector habitats in and around houses are the most effective control measures (Arata et al. 1994). The prevalence of Chagas disease in Bolivia is highest in rural areas, where poverty, lack of education, and poor housing favor infestation by the Triatomid bugs (vinchucas) that carry *T. cruzi*. Baseline surveys (1991) revealed *T. cruzi* sero-prevalence rates in humans ranging from 40 percent to 80 percent in these areas, with 38 percent to 78 percent of the homes infested with the vector, *Triatoma infestans* (Klug, 1834). Over 30 percent of the insect vectors captured in and around the houses were infected with *T. cruzi* (Arata et al. 1994). In Los Tiempos Journal in april 13th in 2003. In Bolivia 1 of 2 boliviens can have chagas'disease and 60% of the country have these disease and 6 of the nine departments. The ideas it was wrong because in the first time, chagas'disease it is not only a problem in rural areas in Bolivie. The chagas'disease is so much important in the great cities too, as Cochabamba, In this city the risk with this infection is so much hight as the rural areas, is increivel that there is in new houses and hight buildings in the center of the city. Although the direct vector infection represents around 82% of all cases(André Medici et. al.2001)

The intense rural-urban and inter-departamental migration in Bolivia show for us the magnitude and its social impact in our country.

Our results show for us that the porcent of infestation in the different homes in the different regions at risk in cochabamba Bolivie were the next: Sacabamba(0,2%9), Anzaldo (2,2%), Tarata (1,0%), Arbieto (2,0%), Aiquile (6,0%), Pasorapa(10,8%), Omerque (14,3%), Mizque (5,4%), Vila Vila (2,9%), Capinota (7,4%), Santibáñez (10,9%), Sicaya (12,7%), Tapacarí (7,5%), Punata (14,3%), San Benito (10,0%), Cliza (3,7%), Tolata (4,3%), Toco (3,2%). The principal results were: Endemics areas (60%), Province with bugs (83), Municipalities with bugs (168), Comunities with bugs (10.321), homes with bugs (700.000), population in risk (4.800.000) seroprevalence(40%), chagásic Cardiopathy (15 a 28%), principal vector(*T.infestans*), Congénital cases (9%).

VE16 - NATURAL PARASITISM OF TRIATOMINAE EGGS AND CONSERVATION IN LABORATORY CONDITIONS

Inturias Alyson, Borda P. Cecilia, Tenorio Vasquez Omar, Torrez Tarqui Alejandra Rocio, Delgado Quispe Gil, Merubia Marcelo, Medrano Mercado Nora,

Inmunoparasitology and Chagas disease Laboratory, Biology Department, San Simón University, Cochabamba-Bolivia

Telenomines are parasitoids of the eggs of other insects in the orders Lepidoptera, Hemiptera, Homoptera, Diptera, and Neuroptera. Some 800 species are presently recognized, but this probably represents at most half of the true number. In our insectary of Inmunoparasitology and chagas'disease laboratory, Biology Department in San Simón Univeristy we maintain a population of the microhymenoptera *Telenomus sp.* endoparasitoid of the eggs of several species of Triatominae (Hemiptera). This insect (*Telenomus sp*.) was inside the eggs of the *Triatoma infestans*, the principal Bolivian vector. Among the 780 eggs examined was found that *T. infestans* egss, were parasitised by *Telenomus sp,* and the temperature that we maintained the telenomus it is in 25 centigrades and 40% atmosphere In 1990 Fernandes et. al. observed parasitoid/egg average was10% in *T. infestans* the present report has special importance considering the real possibility of *Telenomus sp* infestation due to the access of infested triatomine eggs from field captures inducing great damage. These eggs are carefully examined, isolated to study the biology, ecology, and taxonomic are reviewed. Suggestions for greater use of this parasitoid and research needed for improving its biologic control capabilities in the field. Also, the future successful use of *Telenomus sp* to control populations of *Triatoma infestans* and other bugs of the triatominae family. For this our proposal is to do first a strong study inside the laboratory and after we want to go in the little towns in our city Cochabamba and show the effectivite capacity of the telenomus in the biologic control, for this our interested is try to work with some groups interested to study this Hymenoptero. Our city Cochabamba is considered one of the cities in Bolivia, with more infestation,the population at risk consists of the population that lives from 300 to 3.500 meters above sea level, which corresponds to the population in the departments of Tarija, Chuquisaca, Cochabamba, Santa Cruz and part of that of Potosí and La Paz (IDB, 1998). In 1992, the estimate of the population at risk was 3.5 million persons; Although the direct vector infection *T.infestans* represents around 82% of all cases, the intense rural-urban and inter-departamental migration in Bolivia leads to the additional transmission through blood transfusion (15% of the cases). An evaluation carried out in 1994 for Carrasco et. al. showed that the seroprevalence of Chagas in blood banks reaches very high magnitudes in the Departments of Santa Cruz (51%), Tarija (41%), Sucre (39%), Cochabamba (28%), Potosí (24%) and smaller proportions in La Paz (5%) and Oruro (6%).

VE17 - DETERMINATION OF LIPID STORAGE VARIATION IN THE FAT BODY OF *RHODNIUS PROLIXUS* DURING THE DAYS AFTER A BLOOD MEAL

Melo P. L., Pontes E. G., Gonzalez M., Feder D., and Gondim K. C.

Departamento de Bioquímica Médica - ICB – CCS –UFRJ. plmelo@bioqmed.ufrj.br

In the days after a blood meal, *Rhodnius prolixus* addresses large amounts of lipids to the developing ovary where a great number of eggs are produced in relatively short time. The fat body is an organ, which concerns functions of both liver and adipose tissues from vertebrate organisms. When tissue needs are suppressed, during digestion, exceeded lipids are transported to fat body to be stored as triacylglycerol, the main lipid form stored by insects.

The objectives of this work were to determine the variation of lipid storage in fat body during the days following a meal and to analyze the effect of decapitation on this process. Fat bodies from females, males or decapitated males in different days after a blood meal were dissected, lipids were extracted and the amounts of triacylglycerol were determined by TLC. Our results showed that females in the 2º day after blood meal presented the triacylglycerol level of 58 mg / fat body, which increased to around 250 mg / fat body in the 4º day. Triacylglycerol amount was stable until the 13º day, when levels of lipids regularly decreased, reaching about 30 mg of triacylglycerol / fat body in the 20º day. Male storage showed a different profile, increasing gradually until the 10º day, when triacylglycerol was around 460 mg / fat body. After that, levels decreased reaching 200 mg of triacylglycerol / fat body in the 20º day. We can conclude that males store more lipids then females during all digestion cycle. This is probably because males have a metabolic demand smaller than females (responsible for egg production) and also have a slower digestion.

In order to investigate a possible involvement of factors released by head in the accumulation of lipids by the fat body, a few hours after feeding, insects were decapitated and in different days after blood meal the amount of triacylglycerol in the fat body was measured. Results showed that, even without head, insects were able to incorporate and store lipids, achieving the maximal value of 430 mg of triacylglycerol / fat body on the 13º day. Considering these results, we can exclude the possibility of a hormonal factor from head to be involved in the storage of lipids by fat body.

VE18 - CHARACTERIZATION OF LIPASE ACTIVITY FROM THE FAT BODY OF *RHODNIUS PROLIXUS*

Pontes E. G., Mello P. L., Carvalho P.P. and Gondim K. C.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Departamento de Bioquímica Médica - ICB – CCS –UFRJ. e-mail: eguedes@bioqmed.ufrj.br

In insects, the fat body is the main site of storage of lipids and that are accumulated in the form of triacylglycerol (TG). Lipids are transported by lipophorin, a major hemolymphatic lipoprotein, to the different tissues and the interaction of lipophorin with fat body, as well as, midgut and oocytes is mediated by specific binding sites at cell surface. After a blood meal, *Rhodnius prolixus* lipophorin takes up lipids from the midgut and transports them to the fat body. This transfer of lipids is greater in the fourth day after blood meal, as well as, the binding of lipophorin to this organ. During digestion of blood the fat body accumulates lipids and this reserve is maintained at the same level until the thirteenth day, when the amount of lipids decreases. These results suggest that a TG-lipase is probably involved in the control of lipid mobilization in the fat body of *R. prolixus*.

In order to study TG-lipase activity from the fat body of *Rhodnius prolixus*, females ten days after blood meal were dissected and the fat bodies homogeneized. The homogenates were centrifuged at 20,000 x g for 30 min at 4°C and infranatants were used as enzyme source. The infranatants were incubated with radiolabelled triacylglycerol (^{3}H-triolein) in the presence of Triton X-100 and unlabelled triolein, and the amounts of released fatty acids were determined for lipase activity characterization. The optimal concentration of Triton X-100 for determination of lipase activity was 26 mM. The time course of lipase activity was linear for at least 120 min of incubation, suggesting that the enzyme was stable under incubation conditions used. NaF and ATP, known inhibitors of TG-lipase activity, were capable to inhibit the lipase in 30% and 54%, respectively. The study of triacylglycerol hydrolysis is important for the understanding of the process of lipid storage and mobilization in the fat body of *Rhodnius prolixus*.

Supported by CNPq and Faperj

VE19 - BIOLOGICAL PARAMETERS DURING THE OVIPOSITION PERIOD OF *PANSTRONGYLUS MEGISTUS* (BURMEISTER, 1835) (HEMIPTERA-REDUVIIDAE) FEMALES AND EGGS MORPHOMETRY.

[1]Ishihara, F Y; [2]Gil, J L M; [2] Nobrega Filho, M; [1]Rosa, J A da

[1]Departamento de Ciências Biológicas - Faculdade de Ciências Farmacêuticas-UNESP- Rodovia Araraquara-Jaú km 01, 14801-902 Araraquara-SP. [2]Serviço Especial de Saúde de Araraquara.
e-mail: franciscoyi@yahoo.com.br, rosaja@fcfar.unesp.br

Panstrongylus megistus is a Triatominae that has a significant importance in the transmission of Chagas' disease, with *Triatoma infestans, T. dimidiata, T brasiliensis, T. pseudomaculata, T. sordida* and *Rhodnius prolixus* are the main vectors of *Trypanosoma cruzi* in Americas according to Silveira (1985). *P. megistus* is a specie that remains importance in epidemiology because it persist in residual woods as verified by Barata et al (2000) in Araraquara town. However the biological parameters to the oviposition period of *P. megistus* females are unknown. To try to elucidate that, a project was elaborated that evaluated individually five females of *P. megistus*, which had been mated after the 5th instar ecdise. In this summary are presented the partial results referring to two females. For these females had been evaluated: time of oviposition, egg number ranks for female, as well as the variability of eggs size. The length, width and opercular opening diameter of eggshells were mensured by stereomicroscope Leica MZ APO and QWin image analysis system. The statistical analysis was effected using the INSTAT program. The female 1 values in millimeters are: average length 1,909 with standard deviation of 0,12 maximum and minimum values respectively 2,003 and 1,757; average width 1,387 standard deviation of 0,13 and maximum width of 1,459 and minimum of 1,230; average diameter of 0,650 standard deviation of 0,05 with maximum of 0,704 and minimum of 0,596. For female 2 average length 1,882 standard deviation of 0,09 with maximum of 1,936 and minimum of 1,819; average width of 1,341 standard deviation of 0,12 with maximum of 1,457 and minimum of 1,275; average diameter of 0,642 standard deviation of 0,03 with maximum of 0,660 and minimum of 0,621. The two females had different period of oviposition, one for 11 and the other one 12 times in a period of 57 and 61 days. The maximum and the minimum numbers of eggs for the two females occurred in different dates, during the oviposition were perceived great variability in eggshell mensuration. The diameter average values of the female 2 eggshell opening presented a bigger average than female 1, while the length and the width of the eggs of female 1 presented bigger average than the female 2. The female 2 oviposition was bigger than the female 1 and the female 2 presented born nymphs tax of 61,19% while the female 1 was 10,81%.

VE21 - EFFECTS OF THE LIGNAN ISOLATED FROM *PODOPHYLLUM PELTATUM* ON THE DYNAMIC DEVELOPMENT OF *TRYPANOSOMA CRUZI* IN *RHODNIUS PROLIXUS.*

Cabral, MMO[1/2]; Gomes, SAO[3]; Garcia, ES[5]; Azambuja, P[5]; Schaub, GA[4].

[1] Laboratory of Diptera-Department of Entomology- Institute Oswaldo Cruz, Rio de Janeiro. [2] College Pedro II, MEC, Rio de Janeiro. [3] Laboratory of Cellular Biochemistry -Department of Medical Biochemistry- Federal University of Rio de Janeiro. [4] Department of Special Zoology - Ruhr University Bochum, 44780, Germany. [5] Laboratory of Biochemistry and Immunology of Insects- Department of Biochemistry and Molecular Biology, Institute Oswaldo Cruz, Rio de Janeiro.

Trypanosoma cruzi, the causative agent of Chagas disease is transmitted to mammals by hemipteran insects, the triatomines. This flagellate develops as epimastigote in the gut with differentiates to infective form, metacyclic trypomastigotes in the rectum on the invertebrate host. Herein, we described experiments demonstrating different developmental forms of the *T.* cruzi (Dm28c strain) when the insect vector is orally treated with a lignan (Pp) isolated from *Podophyllum peltatum.* The infection of 5th instar larvae of *R. prolixus* were performed by the insects feeding on blood meal containing parasites as controls and parasites plus lignan (Pp) as experimental group.

The observed results were: (i) *in vivo* experiments with *T. cruzi*, comparing the controls with the group treated with 10 mg Pp/ml, showed that in the crop and intestine, no difference in the *T. cruzi* development was observed at days 5, 10, 15 and 20 after infection; (ii) a high significantly accumulation of epimastigotes forms of *T. cruzi* was detected at day 10, 15 and day 20 after feeding, 92%, 84% and 95% respectively in the rectum of treated insects; (iii) in contrast, *in vivo* experiments control group with *T. cruzi* demonstrated that decreased the population of the epimastigotes forms of the parasites, 88%, 10% and 67% respectively, in the rectum of *R. prolixus*; (iv) low numbers of metacyclic trypomastigotes were observed in the rectum treated with the lignan 1 – 3% at 5 to 20 days post infection (p.i.). The control group presented 2 – 21% of the metacyclic trypomastigotes forms of *T. cruzi*; (v) division stages of the parasites were not observed in the rectum of the treated insect. These results suggest that lignan (Pp) of the *Podophyllum peltatum* perhaps affect the metacyclogenesis of *T. cruzi* in this bloodsucking vector insects.

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), DAAD-CAPES, Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ).

VE22 - CHARACTERIZATION OF AN OVARY TREHALASE ACTIVITY IN *RHODNIUS PROLIXUS*.

Santos, R.; Fonseca de Souza, A. L.; Meyer-Fernandes, J. R. and Gondim, K. C.

Departamento de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro, Brasil

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

The eggs of insects accumulate various nutrients during oogenesis to be used as nutrient sources during embryogenesis. Large amounts of macromolecules are synthesized and stored in the oocytes. Proteins and lipids are mainly produced by extra ovarian organs and sequestered by developing ovary. Carbohydrate are taken up by the oocytes and are stored as glycogen. Hemolymph trehalose appears to be the major source for glycogen synthesis, since this disaccharide is the predominant sugar in the hemolymph of most insects. In *Rhodnius prolixus*, we showed that glycogen was accumulated during oocyte growth and utilized during embryogenesis. The major increase in glycogen content occurred when oocytes grew from 1.0 to 1.5 mm in length and, in fertilized eggs, this content decreased after oviposition until 15th day. A trehalase activity was identified in the ovaries of vitellogenic females and in this work we describe the kinetic characterization of this activity. At 7th day after blood meal the ovaries were dissected, washed, homogenized and trehalase activity was determined. This activity was linear with time and protein concentration. Trehalase activity showed a Michaelis-Menten profile and the apparent K_m was estimated to be 1.5 mM. Activity was maximal at pH 4.5 - 5.5 and metal ions had no significant effect on it. Thus, it is possible that this activity is involved in the uptake and hydrolysis of hemolymphatic trehalose by the oocytes to provide glucose for glycogen synthesis, to be used by embryo during its development.

Supported by: CAPES,CNPq, Faperj.

VE23 - LIPID DIGESTION IN *RHODNIUS PROLIXUS* MIDGUT: CHARACTERIZATION OF A TRIACYLGLYCEROL LIPASE ACTIVITY

Grillo L. A. M. and Gondim K. C.

Departamento de Bioquímica Médica, ICB, CCS, UFRJ, Rio de Janeiro, Brasil. e-mail: lagrillo@bioqmed.ufrj.br

One of the major functions of the midgut is to digest dietary lipids, and absorb and process the digestion products for export into the hemolymph. Triacylglycerol is a major lipid component of the diet and the main form for fatty acid storage. The digestive process has been characterized to suggest two models of lipolysis in the midgut lumen, the complete hydrolysis of triacylglycerol to fatty acids and glycerol and the formation of fatty acids and monoacylglycerol. Triacylglycerol lipases are enzymes that preferentially hydrolyze ester links of triacylglycerols and act only on the water-lipid interface. Insect midgut triacylglycerol lipases have been studied in few insects and only in crude preparations. The data suggests that these enzymes preferentially release fatty acid from the 1- and 3- positions, and show a preference for unsaturated fatty acid, and are activated by calcium ions, thus resembling the action of mammalian pancreatic lipases.

In order to study the triacylglycerol lipase activity from the midgut of *Rhodnius prolixus*, two days after blood meal midguts were dissected from adult females and luminal contents was removed. After this, midgut tissue and luminal content were homogenized and incubated with radiolabelled triacylglycerol (^{3}H-triolein) in the presence of Triton X-100 and unlabelled triolein, and the amounts of released fatty acid were determined for the lipase activity characterization. The optimal concentration of Triton X-100 for determination of lipase activity was 0.26 mM and the ratio of molar concentration of triolein to Triton X-100 was calculated to be about 1:130 mM. The time course of lipase activity exhibited linearity for at least 120 min of incubation, suggesting that the enzyme is stable under incubation conditions used. The amount of free fatty acids released was proportional to the amount of homogenate added, thereby indicating that the release of fatty acids was due to lipase activies. The study of triacylglicerol hydrolysis is important for the understanding of the process of lipid digestion in the midgut of *Rhodnius prolixus*.

Supported by CNPq

VE24 - VE-FURTHER INVESTIGATIONS OF LIPID TRANSFER FROM MALES TO FEMALES DURING MATING IN *RHODNIUS PROLIXUS*.

Oliveira,G.A. and Atella,G.C.

Departamento de Bioquímica Médica, Universidade Federal do Rio de Janeiro, RJ, Brasil. E-mail: gial@bioqmed.ufrj.br

Lipophorin is the major lipoprotein in insect hemolymph. It is known to transport lipids of many classes between tissues involved in lipid absorption, storage and utilization. In *Rhodnius prolixus* lipophorin (Lp) transfers lipids to the testis and can be reloaded in fat body and midgut. In this work we are studying the transfer of these lipids from males to females during mating.

Virgin males were injected with Lp labeled in neutral lipid moiety with ^{3}H (^{3}H-Lp) and put together with virgin females. After five days, testis and female spermatheca were dissected and radioactivity estimated. It was demonstrated that ^{3}H-lipids were taken up by male testis and transferred to female spermatheca during mating. When Lp labeled in phospholipid moiety with ^{32}Pi (^{32}P-Lp) was injected, no transfer to females was observed. The ^{3}H-lipids transferred to spermatheca during mating were analyzed by a thin-layer chromatography. Diacylglycerol, triacyglycerol, and free fatty acids were the major lipids found.

To confirm those results, Lp fluorescently labeled in phospholipid moiety with Texas Red phosphatidilethanolamine (TRPE-Lp) and in neutral lipid moiety with Bodipy palmitic acid (Bodipy-FA-Lp) were injected into virgin males. After mating, testis and female spermatheca were dissected and the fluorescence was analyzed by a epifluorescence microscopy (Nikkon Eclipse TE 300). Both fluorescent lipids were visualized in testis but only green fluorescence was found in female spermatheca.

To examine whether the entire Lp particle or only lipids were transferred during mating, Lp was labeled in apolipoprotein moiety with ^{125}I (^{125}I-Lp). Males were injected with ^{125}I-Lp and put together with females. Five days later, no radioactivity was found in female spermatheca.

Supported by CNPq, FAPERJ, PADCT, Pronex.

VE25 - HEME DEGRADATION IN *R. PROLIXUS*: CHARACTERIZATION OF A NEW PATHWAY

Cruz-Oliveira, C.[a]; Paiva-Silva, G.O.[a]; Almeida, I.C.[b]; Oliveira, P.L.[a]; Masuda, H.[a]

aDepartamento de Bioquímica Médica, ICB, CCS, UFRJ, Rio de Janeiro; bDepartamento de Parasitologia, ICB, USP, São Paulo.

Rhodnius prolixus, a Chagas disease vector, presents protective mechanisms against heme-induced oxidative damages generated by the digestion of blood hemoglobin. One of them is the degradation of heme in tissues such as heart and midgut. Heme degradation is well known in mammals where it produces the isomer a of biliverdin IX (BV), carbon monoxide (CO) and free iron. This reaction is catalyzed by heme oxygenase and it‘s highly specific, producing only the isomer a of BV. BV isomers other than the a are widespread among several organisms but their mechanisms of heme degradation are still unknown. We demonstrated that the product of heme degradation in *R. prolixus* is a BV g bound to two cysteine residues. This new BV compound suggests a novel heme degradation pathway. In this work we began the characterization of this new pathway by the identification of its intermediates. Ten days after feeding, females were injected with heme and Sn-protoporphyrin IX. Hearts were dissected after 48 hours, homogenized and analyzed by reverse-phase HPLC. In this conditions, two peaks eluted after *R. prolixus* BV are intensified, suggesting that they correspond to intermediates of this pathway. Curiously, they present high similarity with heme absorption spectrum, suggesting that both have heme in

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

their structure. Purified intermediates were analyzed in an Electronspray Mass Spectrometry for mass determination (ES-MS) or collision-induced fragmentation for structure identification (ESMS/MS). The intermediates have 972 and 794Da. Fragmentation of the ion species corresponding to the intermediates were performed and a specie of same molecular mass than heme was obtained in both cases. The mass difference between the intermediates and heme suggests the addition of two carboxymethylcysteines to the heme molecule before its degradation into BV. Thereafter, these carboxymethylcysteines would be properly processed to cysteines.

Supported by CNPq, FAPERJ,PRONEX, PADCT and HHMI.

VE26 - PRELIMINARY MOLECULAR CHARACTERIZATION OF RETINOIC X RECEPTOR FROM *RHODNIUS PROLIXUS*

Nakamura A[1], Stiebler R[1], Oliveira FB[1], da Silva ICA[1], Furtado DR[1], Fialho E[1,2], Fantappié MR[1], Oliveira MF[1]

1- Departamento de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro. 2- Departamento de Nutrição Básica e Experimental, IN, UFRJ, Rio de Janeiro

Retinoids are a class of compounds derived from b-carotene, which play various roles in biological systems. These compounds have been shown to play a central role in different processes such as cellular proliferation and differentiation as well as their involvement *in vivo* as natural morphogens specifying the antero-posterior axis in vertebrate development. Retinoic acid (RA) is one of the retinoids with morphogenetic activity and this is mediated through interaction with specific nuclear receptors named retinoic acid receptor (RAR) and retinoid x receptor (RXR). Both receptors, in turn, regulate the expression of various genes such as the homeobox (*Hox*), which are involved in patterning specification during development. Little is known about the roles and the enzymes involved in retinoids metabolism in invertebrates. The RXR analog *ultraspiracle* (USP) has been already cloned and characterized in some insects such as *Drosophila*, *Bombyx* and *Aëdes*. Based on the fact that the vertebrate blood is a good source of retinoids and that hematophagous organisms usually ingest large amounts of it to reach their nutritional requirements our group is interested to investigate the effects of retinoid supplementation and the characterization of enzymes of retinoids metabolism in blood-feeding arthropods. The main objective of the present work is to investigate the presence of a putative retinoid x receptor (RXR) in the blood sucking insect *Rhodnius prolixus*. Our first task was to assess whether *R. prolixus* possesses a sequence similar to RXR(USP) genes by southern hybridization of genomic DNA obtained from fat bodies and using the DNA-binding domain (DBD) of the RXR1 of *Schistosoma mansoni* as a probe. Under low stringency conditions a unique band was observed suggesting that *R. prolixus* has a sequence homologous to DBD of RXR. Next, total RNA from the fat bodies of blood-fed adult females of *R. prolixus* were extracted and utilized for cDNA synthesis. PCR reactions were performed by using three different combinations of primers designed to amplify amplicons of 80, 170 and 227bp inside the DBD region of RXR. Three products of 80, 170 and 227bp were amplified and subsequently cloned in a TOPO TA system, indicating that possibly these amplicons correspond to the DBD of *R. prolixus* RXR. Attempts to obtain the sequence of these products are currently underway in our laboratory. In conclusion, the preliminary results presented here suggest that *R. prolixus* contains a putative RXR (USP) which may be involved in important biological roles in this blood-feeding insect.

Supported by: TWAS, FUJB, FAPERJ, CNPq, CNPq (Profix)

VE27 - EFFECTS OF RETINOIDS ON THE OOGENESIS, EMBRYOGENESIS AND MOULT OF *RHODNIUS PROLIXUS*

Stiebler, R.[1], Nakamura, A.[1], Fialho, E.[1,2] Oliveira, M.F.[1]

[1]Departamento de Bioquímica Médica, ICB, UFRJ, Rio de Janeiro. [2]Departamento de Nutrição Básica e Experimental, IN, UFRJ, Rio de Janeiro

Retinoids comprise a class of compounds derived from b-carotene, which the most known is retinal. It has been demonstrated that retinoids are implicated in different biological processes such as cellular proliferation and differentiation as well as their involvement *in vivo* as natural morphogens specifying the antero-posterior axis in vertebrate development. Little is known about the metabolism of retinoids in invertebrates, especially in arthropods. Since the vertebrate blood is a good source of retinoids and that hematophagous organisms, usually ingest large amounts of it to reach their nutritional requirements, the main objective of the present work is to investigate the metabolism of retinoids and their possible role in oogenesis and embryogenesis of the blood feeding arthropods *Rhodnius prolixus*. Blood-fed adult females of *R. prolixus* were injected with 60 pmols of all-trans retinoic acid (at-RA), 60 pmoles 9-cis retinoic acid (9cis-RA) or 5,8 nmoles all trans retinol (at-ROH) in the hemocoel. We observed that at-RA accelerates egg laying, while 9cis-RA delayed egg laying. at-ROH injection did not altered egg laying. The viability of these eggs was decreased in at-RA injected insects while at-ROH did not show any effect. The time of hatching was delayed by both at-RA and 9cis-RA, whilst at-ROH did not modify the time of laying eggs compared to control. When nymphs of 5th. instar were injected with at-RA or 9cis-RA, we observed remarkable changes in the external morphology of these insects such as losses of antennae and legs segments as well as changes in leg morphology. Taken together, these results indicate that retinoids may be exerting some effects in oogenesis, embryogenesis and moulting of blood-feeding insects.

Financial support: TWAS, CNPq, Pronex, HHMI, Faperj, FUJB.

VE28 - POPULATION GENETICS OF *RHODNIUS BRETHESI* IN THE BRAZILIAN AMAZON

Kikuchi SA, Pavan MG & Monteiro FA

Laboratório de Doenças Parasitárias, Departamento de Medicina Tropical, IOC, FIOCRUZ

In the Brazilian Amazon, Chagas disease has traditionally been considered as a disease of wild animals which circulates solely within the sylvatic foci. However, because of deforestation and human colonization of natural ecotopes, Chagas disease is now seen as an emerging disease in this region. Several cases have been recently reported in the Amazon and 10 of the 18 triatomine species that there occur have been shown to be infected with *T. cruzi*. Of particular interest is the transmission cycle that happens in certain areas of the Rio Negro. In these areas, piassava gatherers will leave their homes to go up tributaries of the Rio Negro after certain locations where there is an abundance of the palm tree *Leopoldinia piacaba*. As these locations are usually far from their homes, they may spend months there collecting piassava fibers. During such periods, at night, these workers experience the "attack" of hungry adult *Rhodnius brethesi* that come flying from the piassava palms to bloodfeed.

We are in the process of investigating two issues regarding the triatomine vector species involved in this transmission cycle, *R. brethesi*, by means of mitochondrial DNA sequence analysis: we want to (1) determine the levels of genetic diversity of natural populations (i.e. wild triatomine populations that have never before been exposed to insecticides); and (2) compare different populations in order to determine the degree of genetic structuring and infer levels of gene flow among them.

We have used live bait traps to collected over 200 insects from several piassava

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

palms from six sites along two tributaries of the Rio Negro (Aracá and Padauirí rivers). Geographic distance between individual palm trees sampled ranges from five meters to 200 km. So far 30 insects have been sequenced for a 650 bp region of the mitochondrial cytochrome *b* gene (cyt *b*). Our preliminary findings suggest that, surprisingly, the levels of genetic diversity for these natural *R. brethesi* populations, are extremely low. Among the insects analyzed, 29 share a single haplotype, and one individual has a second haplotype that differs by a single silent substitution. This seems to indicate that these populations have gone through a very recent bottleneck that has drastically reduced the levels of gene variation. We believe that the analysis of a greater number of insects will give us a better understanding of the genetic structure of these *R. brethesi* populations in this area.

Financial support: CNPq

VE29 - EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) IN THE OVIPOSITION AND ECLOSION OF *RHODNIUS PROLIXUS*

Luciana T. Zimmermann*, Evelize Folly das Chagas**, Georgia C. Atella**, Angela H. C. S. Lopes*

*Instituto de Microbiologia Prof. Paulo de Góes, UFRJ; **Departamento de Bioquímica Médica, ICB, UFRJ; Cidade Universitária, Ilha do Fundão, Rio de Janeiro, R.J. 21941-590, Brasil. E-mail: angela.lopes@micro.ufrj.br

Blood-sucking arthropods, especially insects, have been widely used in biological, biochemical, molecular, pharmacological and immunological studies. The main medical interest of these studies is due to the fact that these arthropods may be vectors of virus, bacteria, protozoan and worms that cause emergent and reemerging diseases, like Lyme disease, malaria, leishmaniasis, Chagas' disease and others. The strategy for controlling these diseases is based upon understanding some molecular aspects of vectors and parasites. Platelet-activating factor (PAF) is a phospholipid involved in diverse biological and pathophysiological processes, like cell differentiation, inflammation and allergy. PAF is produced by mammals, other vertebrates, invertebrates, fungi and protozoan. Previous data from our group showed that PAF induces cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*. In the present study we observed the effect of PAF in the oviposition and eclosion of *Rhodnius prolixus*. The insects were artificially fed with rabbit blood in the absence or in the presence of the following modulators: 10^{-6} M PAF, 10^{-6} M WEB2086 (a PAF antagonist) or both 10^{-6} M PAF plus 10^{-6} M WEB2086. There was not significant difference in the number of eggs or in the percentage of eclosion among the four groups of insects. However, a significant number of the eggs from the treated insects led to defective nymphs: 5.6%, 5.3% and 7.7% eggs from PAF-, WEB- and PAF plus WEB-treated insects respectively led to defective first instar nymphs, as compared to the eggs from the control insects, which were all perfect.

Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ and PRONEX (0885).

VE30 - ECDYSTEROID IN DIGESTIVE TRACT OF *RHODNIUS PROLIXUS*

*Feder, D; **Gonzalez, MS.; *Garcia, ES;*Azambuja P. and **Mello CB.

*Department of Biochemistry and Molecular Biology, Oswaldo Cruz Institute, FIOCRUZ, Av. Brasil 4365, Rio de Janeiro, CEP 21045, RJ, Brazil; and **Department of General Biology, Universidade Federal Fluminense, Morro do Valonguinho s/n, CEP 24.001-970, Niterói, RJ, Brazil.

Investigations on the effects of decapitation and azadirachtin treatment on the maturation and organization of the perimicrovillatr membranes (PMM) have been developed in our laboratory. In these studies, we demonstrated that ecdysone is an important hormone to establish *Trypanosoma cruzi* infections and reorganize the *R. prolixus* midgut structures. Herein, we described the results obtained by ecdysteroids radioimmuneassay (RIA) measurements in the stomach (anterior midgut or crop), intestine (small intestine or posterior midgut) and rectum (hindgut) at different days after feeding, treatment with azadirachtin and decapitation of *R prolixus* larvae. The 3 gut compartments were removed from 6 insects, separately homogenized and ecdysteroids extracted with methanol. RIA was performed using ecdysone antiserum, which binds ecdysone and 20-hydroxyecdysone, according to Chang and O' Connor (1979). RIA unit is defined as pg equivalent to ecdysone since this hormone was used as standard.

The main results obtained were: (i) at anterior midgut of control insects ecdysteroids levels began to increase 24 h after feeding and revealed the presence of peak on day 10 after feeding (10 ng/crop); (ii) at the posterior midgut the ecdysteroid peak occurred on day 11 after feeding (12 ng / intestine); in the rectum the peak was of 3 ng/ rectum at day 11 after feeding; (iv) decapitation and azadirachtin treatment drastically reduced the levels of ecdysteroids in the 3 gut compartments during the entire experiment.

Some dates of our lab have shown that *T. cruzi* attachment in the gut is important for both differentiation and multiplication (Garcia and Azambuja, 1991). Gonzalez et al (1999) demonstrated that azadirachtin treatment and decapitation drastically decreases the rate of shedding of the PMM. We now are developing gut cellular cultures to investigate the biosynthesis of ecdysteroids in vitro. The significance of these results will be discussed in relation to the success of the establishment of *T. cruzi* infection in its vector, *R. prolixus*.

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Oswaldo Cruz (Papes) and PADCT. PA; ESG, CBM and MSG are CNPq, and DF is Faperj/ Fiocruz research fellow.

VE31 - VE31 - DOSAGE OF HEMEPROTEINS IN THE SALIVARY GLANDS OF RHODINIINI SPECIES

Artur B. Veloso, Felipe S. Pinto, Nelder F. Gontijo, Marcos H. Pereira.

Departamento de Parasitologia, Laboratório de Fisiologia de Insetos Hematófagos, ICB/UFMG - Belo Horizonte, MG, Brasil

The saliva of the hematophagous insects presents a series of biological activities that interferes not only in the haemostatic mechanism (platelet aggregation, blood clotting and vasoconstriction) as in the inflammatory reaction and the immune response of the host. The nithrophorins are the most abundant salivary proteins in the Rhodniini tribe. These proteins present a heme group, which binds reversibly to the NO. The effects of the nitrophorins are vasodilation, due to the liberation of the NO in the skin of the host, anticlotting and antihistaminic activity. The microdosage of hemeproteins was standardized from the metahemoglobin cyanide methodology based on a clinic test for hemoglobin dosage (Doles reagents ®). The following species were used in the present study: *Rhodnius prolixus*, *R. neglectus*, *R. nasutus*, *R. robustus* and *Psammolestes tertius*. The samples were constituted by the content of four salivary glands diluted in 25mL of water. In the hemeprotein dosage 20 mL of the samples were combined with 200 mL of the Color Reagent (Potassium phosphate 1 mM; Potassium ferricyanide 0.6 mM; Potassium cyanide 0.77 mM and Triton X-100 0.82 mM). After three minutes, 200mL of this solution was transferred to a microplate and read at 550 nm. The standard used for the dosage was equine myoglobin and the results obtained with saliva were expressed as myoglobin equivalents. The results obtained in mg of myoglobin equivalent were: *R. prolixus* (10.96 ± 1,10); *R. neglectus* (14.79 ± 1.07); *R. nasutus* (19.73 ± 4.75); *R. robustus* (36.24 ± 4.34); *P. tertius* (8.54 ± 1.02). In order to corroborate the results further tests will be performed using graphite furnace atomic absorption spectrophotometry.

Supported by: CNPq and FAPEMIG

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

VE34 - *TRYPANOSOMA RANGELI* UPTAKES THE MAIN LIPOPROTEIN FROM *RHODNIUS PROLIXUS*

[a]Folly, E.; [b]Cunha e Silva, N. L.; [c]Lopes, A.H.C.S.; [b]de Souza, W.; [a]Silva-Neto, M. A. C. and [a]Atella, G.C.

[a]Depto de Bioquímica Médica; [b]Inst. de Biofísica Carlos Chagas Filho; [c]Inst. de Microbiologia Geral, ICB/CCS – UFRJ.

Trypanosoma rangeli is a hemoflagellate that employs a wide variety of mammalian hosts and hematophagous insects in its life cycle, including *Rhodnius prolixus*. *T. rangeli*, when infects invertebrate host, penetrates the gut epithelium reaches the hemolymph where they can obtain resources for their metabolism. In insects, lipophorin (Lp) is the major hemolymphatic lipoprotein, which carries and distributes lipids. We have previously demonstrated the Lp uptake by *T.rangeli*. In this work we observed the fate of this lipoprotein in *T. rangeli* epimastigotes. In adition investigate the process of Lp endocytosis in *T. rangeli*.

T. rangeli was incubated with [Texas Red Phosphatidylethanolamine]-Lp for 1h. Parasites were chased in a medium free of fluorescent Lp for different times the fluorescence was analyzed by microscopy. Lp was localized in, anterior region of the cell, i.e. close to the flagellar pocket, and in vesicles at the posterior region. Suggestive that fluorescence observed in parasites was derived from Lp lipids. *T. rangeli* group was incubated with H^3-palmitic acid for 24 hours. After lipid extraction the lipids were analyzed by thin-layer chromatography, followed by plate exposition with phosphoimager screens. We observed that the parasites incorporated radioactivity. The free fatty acids were utilized for *de novo* lipids synthesis. Cholesterol-ester, TG, DG and phospholipids were the major lipids found. The presence of specific Lp receptor in the parasites was determined. *T. rangeli* was incubated at 28ºC or 4ºC, with I^{125}-Lp. Endocytosis of I^{125}-Lp, by *T. rangeli* at 28ºC was higher than at 4ºC, and an excess of BSA did not affect the process. Unlabeled Lp was able to abolish the I^{125}-Lp binding in a concentration dependent manner. These results suggest that *T. rangeli* is able to receive lipids from Lp and Lp uptake is mediate by a specific receptor.

Suported by CNPq

VE35 - SOLUBLE FACTORS WITH ANTIMICROBIAL ACTIVITIES ASSOCIATED WITH THE EGGSHELL OF *RHODNIUS PROLIXUS.*

Bouts, D.M.D.[1]; Furtado, A. P.[2]; Medeiros, L.N.[1]; Kurtenbach, E.[1]; Melo, A.C.A [2]; Silva-Neto, M.A.C.[1]; Masuda, H.[1].

1-Depto de Bioquímica Médica/CCS/ICB/UFRJ; Rio de Janeiro/RJ; 2-Depto de Patologia/CCB/UFPA; Belém/PA

The great evolutive success of the insects can be attributed in part to its capacity of reproducing in a relatively short period time. The egg that is laid in nature has to be protected against mechanical damage and also against microorganisms. The first barrier is physical, and when this barrier is exceeded, diverse chemical factors are set to ensure the elimination of the pathogen. However, the mechanisms of antimicrobial protection associated with eggs are not well known. It was previously described the presence of antimicrobials peptides associated with the exochorion of insects, secreted by the female accessory gland of *Ceratitis capitata* (Marchini and cols., 1997), by the male accessory gland of *Drosophila melanogaster* (Lung and cols., 2001) and by salivar glands of *Pseudacanthoterme spiniger* (Lamberty and cols., 2001). Previous studies, carried out in our laboratory identified an water insoluble protein of 45 kDa, constituent of chorion with antifungal activity. Here, we present the dose dependent inhibition of 45 kDa protein against *Aspergillus niger* AD 102. Besides that we describe here the presence of a water soluble proteins (possibly peptides) associated with the egg, with antifungal activity, of *R. prolixus*. The soluble proteins were extrated by washing the eggs in water or PBS and concentrated. These proteins, extrated from *R. prolixus* eggs were assayed in a microplate with 96 wells containg *A. niger* AD 102 in a Potato-Dextrose medium or incubated on slides into Petri dishes. The results clearly shows the presence of factors capable of inhibiting the growth of fungi. The concentration of 1,25 mg/ml of *R. prolixus* extract inhibited 60% of normal growth in 48 h of incubation. The factors are thermolable.

Supported by: FAPERJ, CNPq, RENOR/CAPES, PNOPG/CNPq.

VE36 - INTERACTION OF *PHYTOMONAS SERPENS* WITH THE SALIVARY GLANDS OF THE INSECT VECTOR *ONCOPELTUS FASCIATUS*

Felipe A. Dias*, André L. S. Santos*, Rosângela M. A. Soares*, Alexandre Romeiro**, Elvira Saraiva*, Thaïs Souto-Padrón*, Mário A. C. Silva Neto***, Antônio F. Pereira* and Angela H. C. S. Lopes*

*Instituto de Microbiologia Prof. Paulo de Góes, UFRJ; **Instituto de Biofísica Carlos Chagas Filho, UFRJ; ***Departamento de Bioquímica Médica, ICB, UFRJ; Cidade Universitária, Ilha do Fundão, Rio de Janeiro, R.J. 21941-590, Brasil. E-mail: angela.lopes@micro.ufrj.br

Some members of the family Trypanosomatidae present great medical, veterinary or economical importance. *Phytomonas* spp are parasites of plants and invertebrates. Some of these species are the causative agents of plant diseases in plantations of economical importance, such as coconut, coffee, oil palm and several fruit. In 1957 Gibbs found flagellated parasite in tomatoes (*Solanum lycopersicum*), which was denominated *Leptomonas serpens* and later *Phytomonas serpens*. However, little is known about the pathogenicity of *P. serpens* for either plants or insects. Dipteran and hemipteran insects are involved in the transmission of trypanosomatid parasites of plants. The phytophagous hemipteran insect *Oncopeltus fasciatus* is the natural host of *Phytomonas elmasiani*. The colonization of the salivary glands of the vector is a major event in the life cycle of *Phytomonas* spp. In the present work, the salivary glands of *O. fasciatus* were extracted from the bodies and the dissected parts were washed with PBS pH 7.2 previously to the interaction assay. The parasites were harvested and washed with PBS pH 7.2. These protozoans were allowed to interact with the dissected salivary glands. In this work parasites of the species *P. serpens* adhered tightly to cells of the dissected salivary glands of *O. fasciatus*. Taking this result into account, we decided to study the molecular aspects of this interaction. Total protein extract of *O. fasciatus* was separated by SDS-PAGE and transferred onto a nitrocellulose sheet, which was exposed to sulfo-NHS-biotin-labeled *P. serpens*. These labeled parasites were able to bind to a protein of 130 kDa, present in the protein extract of the *O. fasciatus* salivary glands. Further experiments are required aiming the purification and identification of this protein.

Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ and PRONEX (0885).

VE37 - PHYLOGENY AND POLYMORPHISM OF THE *KERTESZIA* SUBGENUS ASSESSED BY THE SECOND INTERNAL TRANSCRIBED SPACER (ITS2) OF RDNA.

Malafronte, R. S1 2; Marinotti, O2 3; Marrelli, M. T2; Tubaki, R4, Curado, I4 and Nassar, M. N1 5.

1- Instituto de Medicina Tropical, Lab. de Protozoologia; 2- Instituto de Ciências Biomédicas, Depto. de Parasitologia; 3- University of California Irvine, Department of Molecular Biology and Biochemistry; 4- Superintendência de Controle de Endemia, SUCEN; 5- Universidade Mackenzie, FCBEE.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Kerteszia is a small subgenus that includes neotropical Anopheles species. Some of these species, such as *An. bellator*, *An. cruzii* and *An. homunculus*, can be sympatric and vectors of human and monkey malaria in Southeastern and Southern Brazil. Another species, *An. laneanus*, has been suspected to be a malaria vector in Bolivia. The identification of *An. cruzii* and *An. homunculus* specimens, based on morphological characteristics, is troublesome. In order to evaluate molecular tools to assess the polymorphisms and phylogeny of the *Kerteszia* malaria vectors we cloned and sequenced their ITS2 and compared them with data obtained from other anophelines.

Adult females (*An. cruzii*, *An. bellator*, *An. homunculus*) and larvae (*An. laneanus*) were collected in the State of São Paulo and identified. DNA extraction, PCR amplification, DNA cloning and sequencing, were performed as previously described (Malafronte et al., 1999). Sequence data were aligned using the CLUSTAL W (1.60) and the phylogenetic trees were constructed by MEGA (Molecular Evolutionary Genetics Analysis ver. 1.01, Kumar et al. 1993).

Analysis of the sequences showed ITS2 regions of those anophelines with lengths varying from 332 to 354 nucleotides and their GC contents varied from 53 to 62,5%. Comparing to *An. cruzii*, the major vectors of Atlantic Forest, the alignment of the ITS2 sequences showed divergences such as 0,5% *An. cruzii*/ *An. laneanus*, 1,5% *An. cruzii*/*An. bellator* and 9,5% *An. cruzii*/*An. homunculus*. Comparisons of the ITS2 sequences also showed differences between the sibling species *An. cruzii* and *An. homunculus*, which can be used as tools for the identification, at the molecular level, of these species.

VE38 - MORPHOLOGICAL ANALYSES OF *ANOPHELES (NYSSORHYNCHUS) AQUASALIS* INFECTED WITH *PLASMODIUM (PLASMODIUM) VIVAX*.

Ferreira, M.A.P. and Pimenta P.F.P.

Laboratory of Medical Entomology, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Fiocruz-MG, Brazil. E-mail: pimenta@cpqrr.fiocruz.br

The human malaria has been considered a serious problem of health in the world. The interaction studies involving New World vectors of the human *Plasmodium* are still very limited. Structural and ultrastructural analysis was accomplished with the objective of knowing the normal aspect and the migration of the *P. vivax* in the *A. aquasalis* midgut. Histological techniques, confocal laser, transmission and scanning electron microscopy were done in order to study the organization of the cellular types, the cell ultrastructure, the kinetics of the modifications of the midgut when blood fed and the establishment of the infection for *P. vivax* following the cycle in the mosquito vector *A. aquasalis*. The alimentary tract of mosquitoes is generally divided into three regions: foregut, midgut and hindgut. The foregut and the hindgut have ectodermal embryological origin, distinctly from the midgut, which is originated from the endoderm. The midgut itself can be divided into two topographic regions according to their location: thoracic and abdominal midguts. The histology showed the midgut composed by a columnar single epithelium of microvillar cells supported by a basal membrane. We observed in both areas of the midgut, a cellular heterogeneity as for the afinity for the staining revealed mainly by the presence of acid components. The external and internal aspects of the midgut presented a regular surface. Secretory vesicles were found frequently in the cells of the thoracic region; the abdominal region, besides revealed cells with vesicles, also showed frequently organelles related with the protein's synthesis. The cytochemical aspects of the apical and basal surface demonstrated the presence of anionic components, as well as, of the carbohydrates. The *P. vivax* infection was determined by the presence of oocysts, which were considered young when they showed sporoblastoid center, organization of sporozoites and irregular wall; mature oocyst presented flat surface and disorganized sporozoites inside. We visualized the escape of the sporozoites from the oocyst, the liberation to the hemocel and the invasion of the secretory cells in the salivary gland of the *A. aquasalis*, which were not synchronically after the infection. Throughout the confocal laser microscopy, we observed ookinete into the epithelium and preferentially in the intercellular space, without to demonstrate alterations in the cytoskeleton, with little modification in the microvilli. The ookinete migrate to the basal portion of the epithelium, where the oocysts modify themselves. This work is showing the details of morphological observations of the interaction using human *Plasmodium* in a New World vector.

Financial Support: CNPq, Fapemig and Fiocruz.

VE40 - BIOCHEMICAL CHARACTERISATION AND PARTIAL PURIFICATION OF A INTESTINAL α-GLUCOSIDASE AFTER SUGAR FEEDING OF THE BRAZILIAN MALARIAN VECTOR MOSQUITO, *ANOPHELES AQUASALIS*.

Souza-Neto, J. A.[1], Lima, J. B. P.[2*], Valle, D.[2*], and Ribolla, P. E. M.[1]

[1]Laboratório de Entomologia Molecular, Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista - Rubião Júnior, Botucatu, São Paulo, Brasil, 18618-000. [2]Laboratório de Transmissores de Hematozoários, Departamento de Entomologia, Instituto Oswaldo Cruz - Fiocruz, Av. Brasil 4365, Rio de Janeiro, RJ, Brasil. 21045-900. *Laboratório de Entomologia, Instituto de Biologia do Exército,Rio de Janeiro, RJ, Brasil.

The main source of sugar in nature is the nectar, which is composed by the most abundant dissacharide, sucrose. Mosquitoes, in general, feed constantly on sugar, once the digestion of this substance is very important due to its hole on supplying the energy required for female ovarian maturation and wings beating, which affect directly the vectorial capacity of these insects. The hydrolysis of the bounds between a carbohydrate and anyother molecule is carryed out by the glycosidases, and within this group are the a-glucosidases, which may cleave the bounds Glc a-1,2; a-1,3; a-1,4e a-1,6. These enzymes have been already described in midgut and salivary glands of flebotomines, however, in mosquitoes, a-glucosidases activities have been only detected in salivary glands. Molecular analysis have shown two genes that encodes to this enzyme in the midgut of the mosquito *Anopheles stephensi*, but activity assays have not still been done. In the current study, we have identified at least one a-glucosidase after female feeding with 10% sucrose. Others glycosidases have shown not to be present when only sucrose is given to the mosquitoes. Our data have shown that a-glucosidases are present in the female midgut even before feeding of these mosquitoes with sucrose, and it is slightly activated after that. Biochemical characterization has been performed, and the enzyme is present in the midgut, in both soluble and insoluble extracts. The major portion of the activity has been found associated with the microvilli cells, in according to the exohydrolases properties. The optimal pH is around 5.5, and the enzyme is sensitive to the presence of Tris, even though in low concentrations (2 mM). The a-glucosidase activity is also affected by the incubation of the enzyme at 45 ºC. The enzyme has been partialy purified by molecular exclusion (Superdex 200 HR 10/30) and hidrophobic interaction (Resource Phe) cromatographies. In parallel, the polypeptide sequences of a-glucosidases from some insects have been aligned, and degenerated oligonucleotides are going to be constructed, based on the conserved sequence regions, for the gene characterization.

Supported by: FAPESP

VE41 - HEME DEGRADATION IN THE MOSQUITO *AEDES AEGYPTI*

Pereira, L. O. R.; Paiva-Silva, G. O.; Dunkov, B. and Oliveira, P. L.

Departamento de Bioquímica Médica, ICB - CCS - UFRJ - Rio de Janeiro –

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Brasil, Department of Biochemistry and Molecular Biophysics, University of Arizona, USA

In hematophagous animals, blood digestion results in the release of peptides, amino acids and the hemoglobin prostetic group heme; a molecule with a high oxidative potential. The independent evolution toward a common set of problems, related with the oxidative stress, has led to some quite independent solution or strategies to deal with heme, in these animals. In some organisms, many of these mechanisms have been described. In *Rhodnius prolixus,* for example, heme is degraded in the midgut epithelium and heart, producing a green pigment which is a modified biliverdin, different from the one produced by the enzyme heme oxygenase present in mammals, plants and bacterias. In *Aedes aegypti*, the vector of dengue and yellow fever viruses, it is known that heme other anti-oxidative defenses were described, as the presence of anti-oxidative enzymes and the peritrophic matrix that has a role in haem binding. In this work, we intend to study the heme degradation mechanism in the mosquito *A. aegypti.* and in a mutant of *Anopheles quadrimaculatus larvae*.

During blood digestion, a large amount of a green pigment is produced and secreted to the intestinal lumen of this mosquito. We have been trying to investigate the chemical nature of this molecule by a liquid chromatography analysis in reverse phase with a C18 column in HPLC, with an acetonitrile gradient (5% to 80%) in a trifluoroacetic acid 0,05% solution. We've compared this pigment with biliverdin and, specially, with the one produced by *R. prolixus*.

It has been shown that *R. prolixus* biliverdin has a higher hidrophobicity comparing with gama-biliverdin. Preliminary analysis showed that *A. aegypti* pigment seems to be more hydrophobic than the *R. prolixus* biliverdin. Additionally, the spectrum of *A. aegypti* pigment is very similar to gama-biliverdin spectrum.

Considering the different chemical nature of these pigments, that reflects divergent haem degradation vias, one can conclude that the insects developed different strategies against blood-feeding challenge.

VE42 - *AEDES AEGYPTI* HEMOLYMPH PROTEINS AND ANTI-DENGUE GENES.

Feitosa, F.M., Burini, B., Domingues, A., Chinoca, J. & de Lara Capurro, M.

Depto Parasitologia, ICB – USP – fabiana@icb.usp.br

We are developing transgenic mosquitoes that are resistant to dengue virus to test the hypothesis that genetically-engineered vectors can be used to block transmission of the disease. The focus of our research project is the study of interactions between the dengue virus and its mosquito vector. Conventional approaches to disease control such as drugs, insecticides, or vaccines have been thus far ineffective. Our research aims to understand the molecular mechanisms of virus development and interference with the mosquito host. This research will facilitate the development of new control measures through genetic manipulation of mosquito vectorial capacity.

Recombinant monoclonal antibodies (scFv-Mab; *s*ingle *c*hain *F*ragment IgG *v*ariable region *M*onoclonal *anti*body) are powerful weapons for blocking vector-borne diseases. We have already successfully developed and expressed the variable portion of a Mab (N2-scFv) that prevents *Plasmodium gallinaceum* sporozoites invasion of mosquito salivary glands (de Lara Capurro et. al., 2000). We are currently developing five recombinant scFv-Mab´s that recognize all four dengue virus serotypes. These scFv-Mab´s will be used to test if they can be expressed in transgenic mosquitoes to block virus transmission. The corresponding heavy- and light-chain variable regions encoding the anti-dengue 1A10-2 Mab, 1B7 Mab, 2H2 Mab, 9A Mab and 3H5 Mab were engineered to produce single-chain antibody constructs, 1A-scFv, 1B-scFv, 2H2-scFv, 9A-scFv and 3H5-scFv. We are at the final steps to test the ability of these recombinant antibodies to block transmission in a transient expression system. Moreover, they can provide a valuable tool in the search for the mosquito receptor(s) for dengue viruses.

As a second goal from our research we are studying the expression of hemolymph proteins in dengue infected mosquitoes. To start this approach fat bodies libraries were obtained from females sugar fed and 24 h after blood feed. We start a EST catalog of these libraries. The partial data show that from 200 clones analysed. We have 106 contigs and from these contigs we have at the least 65 new putative proteins.

Supported by FAPESP

VE43 - HYDROGEN PEROXIDE DETOXIFICATION BY CATALASE IN THE MIDGUT OF *AEDES AEGYPTI* AND ITS MODULATION BY HEME

Oliveira, J.H.M.C.[1]; Alvarenga, P.H[1]. and Oliveira, P.L.[1]

1-Departamento de Bioquímica Médica, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro – RJ, Brasil, CEP 21910-590

Aedes aegypti females ingest large amounts of vertebrate blood in a single meal. The hydrolysis of hemoglobin in the midgut lumem releases huge amounts of heme, its prosthetic group. Since free heme can generate reactive oxygen species, which are able to oxidize lipids, nucleic acids and proteins, this blood-sucking mosquito is subjected to an oxidative chalange imposed by blood feeding. To counteract this heme toxicity *Aedes aegypti* possesses antioxidant mechanisms that make possible its survivor, among these is catalase, an antioxidant enzyme that detoxifies hydrogen peroxide into water and oxygen. The importance of this reaction is to avoid hydrogen peroxide interaction with heme or iron leading to hydroxyl radical formation, the most toxic oxygen radical.

Once blood is a source of oxidative stress we fed *Aedes aegypti* with blood, plasma or plasma plus hemoglobin. Different hours after the meal (0, 12, 24, 36 and 44 hours) the peritrophic matrix and the midgut epithelium were dissected, homogenized and catalase activity was measured according to Aebi (Aebi et al., 1984). In the epithelium, this activity showed the same profile for the three kinds of meal, reaching its maximum among 24 – 36 hours after the meal, but was greater in mosquitoes subjected to blood and plasma plus hemoglobin than in mosquitoes subjected to plasma feeding. In the peritrophic matrix we observed a small catalase activity during all the digestive process in mosquitoes fed with plasma or plasma plus hemoglobin. However, in mosquitoes fed with blood this activity was higher 12 and 24 hours after meal.

We inhibited catalase activity from mosquitoes fed with rabbit blood *in vitro* and *in vivo* using different concentrations of 3-amino-1,2,4-triazole, a specific catalase inhibitor. The *in vitro* inhibition profile of the enzyme present in peritrophic matrix was different from the enzyme present in midgut epithelium, indicating that they are different. Probably, the enzyme from the peritrophic matrix is provided by the rabbit blood since its *in vitro* inhibition profile is quite similar to the rabbit blood inhibition profile.

To perform the *in vivo* inhibition assays we fed mosquitoes with blood plus different concentrations of 3-amino-1,2,4-triazole. The *in vivo* inhibition profile from the midgut epithelium was similar to the observed in the *in vitro* experiments. Nevertheless, catalase present in the peritrophic matrix was not inhibited. Since inhibition of catalase by 3-amino-1,2,4-triazole is dependent on hydrogen peroxide, we expect that hydrogen peroxide produced in the epithelial cells doesn't diffuse to the peritrophic matrix, but this hypotesis needs to be tested.

Supported by: HMMI, Gorgas Memorial Institute, Faperj, Pronex, CAPES, PADCT and CNPq.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

VE44 - *AEDES* HEMOLYMPH PROTEINS EXPRESSION DURING *PLASMODIUM GALLINACEUM* OOCYST FORMATION.

ARAUJO, R.V.; MIRANDA, C.M., BURINI, B.C., BIANCHI, A.G. de & De LARA CAPURRO, M.

Universidade de São Paulo – Instituto Ciências Biomédicas II – Depto. de Parasitologia.

The quantity of vitellogenin (VG), ferritin (FE), lipophorin (LP), lipophorin receptor (LPR), vitellogenin receptor (VGR), transferrin (TF) and carboxypeptidase (CBX) transcripts was measured in *Aedes aegypti* following infection with *Plasmodium gallinaceum*. *Aedes* females were infected with *Plasmodium* by feeding upon infected chicken. After 6 days the females laid its eggs and a second blood meal in a non-infected chicken was taked by the infected *Aedes* females. The total RNA in infected females showed a lower level, when compared to control non-infected females, 24 hours after second blood meal (2^{nd} BM) in carcass and at 48 h (2^{nd} BM) in ovaries.

A lower level of VG mRNA also occurred 24 h (2^{nd} BM). However, by 48 h (2^{nd} BM), higher levels of VG transcripts were detected in fat bodies. The levels of VGR and LPR transcripts showed significant reductions in ovaries. Nevertheless, by 24 h (2^{nd} BM), CBX transcripts were accumulated in fat bodies.

The levels of LP, TF and FE showed no differences between infected and non-infected females. These results suggest that vitellogenesis is affected by *Plasmodium* infection on *Aedes aegypti*.

Supported by FAPESP. R.V.A. is a CNPq fellowship.

VE45 - *AEDES AEGYPTI* MIDGUT IS AFFECTED BY *BLASTOCRITHIDIA CULICIS* COLONIZATION

Corrêa-da-Silva, M.S.[1], Lessa, L.P.[2] Fampa, P.[2] Lima, D.C.[3] Saraiva E.M.B.[2] and Motta M.C.M.[1]*

1-Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho/UFRJ; 2-Laboratório de Imunobiologia das Leishmanioses, Instituto de Microbiologia Prof. Paulo Góes/UFRJ; 3-Departamento de Entomologia, Instituto Oswaldo Cruz, Rio de Janeiro/RJ.

The mosquito *Aedes aegypti* is an important vector of human diseases such as dengue, yellow fever and filariasis. The interaction of monoxenic trypanosomatids with hematophagous insects acquired more importance after descriptions of opportunistic infection of currently non-pathogenic trypanosomatids in humans with immunodeficiency and also in immunocompetent patients. The mosquito midgut is the first tissue that pathogens interact and is formed by a single layer of columnar epithelial cells with two distinct morphologies: densely microvillated cells, that are predominant, and less microvillated cells with electron-lucent cytoplasm. Ultrastructural studies of the midgut invasion by *Plasmodium gallinaceum* showed that ookinetes interact with epithelial cells and induces morphological changes as cell swollen, surface blebs and vesicles development. Previous studies have proposed that *Plasmodium* ookinetes invade a specific cell type which do not stain with basophilic dye and is less osmiophilic. Differential interference contrast microscopy showed that ookinetes invade a midgut cell at the lateral apical surface and might lyse it. The mechanism that results in death of invaded midgut cell, necrosis or apoptosis, is not yet clear.

In this work we analyzed by scanning and transmission electron microscopy the colonization of *Aedes aegypti* midgut by *Blastocrithidia culicis*, an endosymbiont-bearing trypanosomatid, which is monoxenic and presents typical features such as a modified cytoskeleton and different surface properties. In this work we extend previous observations of our group showing that *B.culicis* was the endosymbiont-bearing trypanosomatid species which better interacted with explanted midguts of *A. aegypti*. We demonstrated that *B. culicis* was able to survive and multiply in *A. aegypti* guts for >30 days after in vitro feeding. Ultrastructural analysis by scanning electron microscopy evidenced that *B. culicis* interacts with microvillated columnar cells initially via flagellum, then protozoa are observed inserted in the microvilli with part of the cell body. The microvillated columnar cells revealed bare bodies protruding and being released to the gut lumen. Although these cells present a normal cytoplasm electrondensity by transmission electron microscopy, the released bare bodies showed an electron-lucent aspect. The disturbance in the aspect of gut epithelia seems to be associated to the protozoa presence, since it was not observed in uninfected midguts. Protozoa did not penetrate epithelial gut cells and haemocoele invasion was not observed, although destruction of columnar cells may suggest this possibility.

Taken together, results provided by this work are interesting for comparative studies involving pathogens-mosquito models, since *B. culicis*, a non-pathogenic trypanosomatid, promoted an epithelial response in *A. aegypti* midgut similar to that observed for *Plasmodium*.

This work was supported by: CNPq, FAPERJ, FUJB AND PRONEX.

VE46 - XANTHURENIC ACID (XA): A MAJOR MOLECULE IN THE GUT PHYSIOLOGY OF *AEDES AEGYPT*

Vitor LA Lima [1], Luiza OR Pereira [1], Ivan JF Peter da Silva [1], Gabriela Paiva-Silva [1], Viviane M Bernardes [1], Igor C. Almeida [2] and Pedro L Oliveira [1]

1 Departamento de Bioquímica Médica, UFRJ. 2 Departamento de Parasitologia USP

Xanthurenic acid (MW 205.2 g/mol) is a tryptophan derivative that was recently (1998) characterized as being the exflagellation factor for the malaria parasite. XA shows a preeminent coordination chemistry (it binds Ca^{2+}, Fe^{2+} and heme). In addition, XA can protect soluble proteins against OH* damage. XA is present in larvae and in saliva of mosquitoes but until now nothing was proposed about what is its function in the mosquito physiology.

In this work, measuring by HPLC, the XA content (identified by it's absorption and mass spectra) of the gut during de course of blood digestion, we are showing that XA is present in the gut in a concentration about ten milimolar. The time course of XA concentration during digestion shows a peak in 24 hours (coinciding with the peak of free heme). Furthermore, we observed that XA can protect azolectin (a soybean phosphatidylcholine-rich fraction) from heme-catalyzed oxidation.

Taken together, our results suggest that XA is an important molecule in the gut physiology of *Aëdes*. It is present in a high concentration in the digestive scenario and interacts with all of the other principal characters: heme, calcium, iron and free radicals. The time course of XA concentration, with a peak at 24 hours after blood meal, indicates that XA is synthesized by the intestinal epithelium, but not derived from the ingested saliva.

VE47 - CHARACTERIZATION OF BINDING OF HEME TO THE *AEDES AEGYPTI* INTESTINAL MUCIN – 1 (AEIMUC1)

[1]Alvarenga, P.H.;. [2]Devenport, M.; [1]Bianconi, M. L.; [2]Jacobs-Lorena, M and [1]Oliveira, P.L.

1-Departamento de Bioquímica Médica, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro – RJ, Brasil, CEP 21910-590. 2- Department of Molecular Microbiology & Immunology, Johns Hopkins University. Bloomberg School of Public Health - Baltimore, Maryland 21205. E-mail: hessab@bioqmed.ufrj.br

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

The gastrointestinal tract of adult female mosquitoes possesses an extra cellular structure, the peritrophic matrix (PM), which surrounds the ingested blood and separates it from the epithelial cells of the midgut. We have shown previously that a large amount of heme (a pro-oxidant molecule) is generated during blood digestion, and that this is bound by the PM and subsequently excreted with the feces (Páscoa, V. et al., 2002). This suggests that PM may act as an antioxidant defense and a heme detoxification mechanism.

Little is known about the proteins present in *Aedes aegypti* PM. The first protein described, called *Aedes aegypti* Intestinal Mucin-1 (AEIMuc-1), is a mucin-like protein induced by metal feeding in both larvae and adult, and by blood feeding in adults (Rayms–Keller, A. et al., 2000). Here we show that this protein (herein called B1) is capable of binding large amounts of heme and is a potential candidate to be involved in the role of PM in the adaptation of this insect to blood feeding.

This protein is composed of 3 chitin-binding domains and one mucin-like domain. Deletion constructs were made in order to identify which domains are important for binding heme and assayed using calorimetric and spectrophotometric titration. All constructs tested were capable of binding heme, but with different stoichiometries. The whole protein was capable of binding up to 12 heme molecules per polypeptide chain, 9 of which had absorption spectra similar to the so-called regulatory heme-binding domains, attributed to cysteine and proline motifs. Cysteine-proline motifs are also present in the chitin binding domains of B1 were they form intramolecular disulphide bounds and allow interaction with chitin. Calorimetric assays also indicated that there is more than one type of heme binding site (apparently 3) with different affinities, and indicated that there is a different kind of event happening after saturation of binding sites, suggesting the formation of an aggregate. Together with the fact that B1 was unable to prevent heme toxicity this data suggests that this protein may be acting as a heme aggregation nucleation centre, but this hypothesis needs to be tested.

Circular dichroism spectra were performed in order to investigate whether bound heme changes the secondary structure of this protein. Results indicated that both the whole protein and the constructs have a secondary structure mainly composed of random coil and that bound heme didn't lead to conformational changes.

Supported by: HMMI, Gorgas Memorial Institute, Faperj, Pronex, CAPES, PADCT and CNPq.

VE48 - APPLICATION OF A NEW SURVEILLANCE METHOD OF *AEDES AEGYPTI* IN BOTUCATU, SÃO PAULO.

Nogueira, L.A., Gushi L.T., Ribolla, P.E.M.

Laboratório de Entomologia Molecular – Departamento de Parasitologia – Instituto de Biociências – Universidade Estadual Paulista - Campus de Botucatu – São Paulo – Brasil.

The World Health Organization (WHO) keeps searching for effective means on dengue control. The main problem relates to control of the major vector, *Aedes aegypt*i. Currently in Brazil, the control program applied by Fundação Nacional de Saúde/ Ministério da Saúde (FNS/ MS) in *Aedes albopictus* and *Aedes aegypti* populations are larval surveys. There are many problems that compromise the reliability of this technique. An interesting alternative is the using of a new control strategy named PCI (Premise Condition Index). This model relates the property condition, like house condition, yard condition, and degree of shade; to the occurrence of *Aedes* spp. oviposition. The PCI validation is achieved by association for the three property variables (house, yard and shade) calculated in scores from 3 to 9. The lowest scores point to properties in good conditions and unfavorable breeding environment. In opposite, the highest scores prove high risk properties to *Aedes* spp. infestation. The present study is based on the application of PCI in properties located in the urban area of Botucatu to confirm the effectiveness of this new tool. With the support of SUCEN (Superintendência de Controle de Endemias) and Secretaria de Saúde de Botucatu, ovitraps have been set in 105 spots all over the city and their properties qualified. Results showed that 64,7% of properties with scores 8 and 9 were positive to *Aedes albopictus* while only 19% of properties with scores 3 and 4 were positive. This preliminary analysis has demonstrated the accuracy of PCI method, since the major occurrence of *Aedes* spp. has been observed in properties with highest scores. The analysis for *Aedes aegypti* has been not significant due its low incidence during the survey. New surveys will be done in order to expand the results.

VE49 - PRESENCE OF CHITINASE ACTIVITIES IN THE GUT OF *AEDES AEGYPTI* (DIPTERA:CULICIDAE) LARVAE FOR DIGESTION OF CHITIN-RICH STRUCTURES.

Souza-Neto, J. A.[1], Gusmão, D. S. [2], and Lemos, F. J. A.[2]

[1]Laboratório de Entomologia Molecular, Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista - Rubião Júnior, Botucatu, São Paulo, Brasil, 18618-000. [2]Laboratório de Biotecnologia, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense. Av. Alberto Lamêgo 2000, Campos dos Goytacazes, Rio de Janeiro, Brasil. 28015-620.

Mosquito larvae are believed to be capable of digesting chitin, an insoluble polysaccharide of N-acetylglucosamine, for their nutritional benefit. Studies based on physiological and biochemical assays were conducted in order to detect the presence of chitinase activities in the guts of the detritus-feeding *Aedes aegypti* larvae. Larvae placed for 24 h in suspensions of chitin azure were able to digest the ingested chitin. Semi-denaturing PAGE technique using glycol chitin and two fluorogenic substrate analogues showed the presence of three distinct chitinase activities: an endochitinase that catalyzed the hydrolysis of chitin; an endochitinase that cleaved the short substrate [4MU$(GlcNAc)_3$], and an exochitinase that hydrolyzed the chitobioside [4MU$(GlcNAc)_2$]. The endochitinase had an extremely broad pH-activity against glycol chitin and chitin azure, ranging from pH 4.0 to 10.0. When the substrate [4MU$(GlcNAc)_3$] was used, two activities were observed at pH ranging from 4.0 to 6.0 and 8.0 to 10.0. Chitinase activity against [4MU$(GlcNAc)_3$] was detected throughout the gut, displaying the highest specific activity in the hindgut. The pH values of the gut content were determined with the color shift indicators after larvae feeding. A correlation was observed between pH measured in guts of feeding larvae (pH 10-6.0) and pH of activity of the gut chitinases. Considering the obtained data, it is possible to postulate that gut chitinases may be involved in the digestion of the ingested chitin-containing structures, and in partial degradation of the chitinous peritrophic matrix (or membrane) in the hindgut.

Supported by: FENORTE – CNPq – WHO/TDR

VE50 - *BOOPHILUS* YOLK CATHEPSIN (BYC), AN ASPARTIC PROTEASE FROM *B. MICROPLUS* EGGS THAT LACKS THE SECOND ASP-RESIDUE FROM ITS CATALYTIC SITE

Nascimento-Silva, M.C.L.[1]; Silveira, A B[1].; Silva-Neto, M. A C[1].; Juliano, L[2].; Oliveira, P.L.[1] and Sorgine, M.H.F.[1]

1- Departamento de Bioquímica Médica, ICB - CCS - UFRJ - Rio de Janeiro - Brasil. 2- Departamento de Biofísica, UNIFESP/EPM - São Paulo - Brasil

The hard tick *Boophilus microplus* is a bovine ectoparasite responsible for great economical losses in tropical and subtropical areas. In Brazil, the annual losses due to this tick may reach 268 million dollars. In order to solve these problems, new effective control methods must be developed.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

Two aspartic proteases were previously characterized in our laboratory from *B. microplus* eggs, named BYC and THAP (Logullo, 1998, Sorgine 2000). *Boophilus* yolk cathepsin (BYC) was tested as a potential component of a protective vaccine against this tick, being able of inducing an immune response in cattle (da Silva Vaz, 1998).

Recently BYC was cloned by RT-PCR and the analyses of its amino-acid sequence demonstrated great similarity with other aspartic proteases. In spite of this similarity, BYC's sequence shows many important differences in the putative active site of the enzyme. The most important one is the lack of the second Asp residue, highly conserved in this class of protease. Although the classical mechanism for aspartic proteases catalysis requires both Asp residues for the nucleophilic attack to the peptidic bond, BYC was shown to be active against haemoglobin (Hb), tick vitellin (VT) and synthetic substrates. Since BYC was proteollyticaly active (although lacking an important part of it's active site) we believe that BYC might have a catalytic mechanism different from the other aspartic proteases.

Sequences predictions of BYC secondary and tertiary structure were made using Swiss Prot database. BYC is a Beta-sheet protein as confirmed by Circular Dichroism analysis. The sequence alignments of BYC and Renin showed a high conserved secondary structure identity, making possible to create a molecular model of BYC's tertiary structure and analyse its active region closely, so as to determine the residues missing in the active site.

As vitellin proteolyses is being show to be controlled by fosforilation in *B. microplus* eggs, we decide to investigate if BYC had fosforilated amino-acid residues by Western blot. The assays were positive for tyrosine residues but not for serine residues. We are now testing the effects of defosforilation on enzyme activity.

Supported by: HHMI, Gorgas Memorial Institute, Faperj, Pronex, CAPES, PADCT and CNPq

VE51 - PARTICIPATION OF *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE) AND *CTENOCEPHALIDES FELIS FELIS* (SIPHONAPTERA: PULICIDAE) IN THE EPIDEMIOLOGY OF CANINE VISCERAL LEISHMANIASIS

Maria Teresa Zanatta Coutinho, Lilian Lacerda Bueno, Annelise Sterzik, Ricardo Toshio Fujiwara, Evaldo Nascimento, Edilene Matias do Amaral, Débora Cristina Carline Alvarez, Odair Genaro "*in memoriam*" e Pedro Marcos Linardi.

The vectorial competence of the tick *Rhipicephalus sanguineus* and the flea *Ctenocephalides felis felis* is discussed in relation to the epidemiology of canine visceral leishmaniasis, taking into account its strict association with dogs and the low indices of natural infection presented by its known vector, the phlebotomine sand fly *Lutzomyia longipalpis*.

To determine the viability of the parasites in *R. sanguineus* and *C. felis felis*, 72 hamsters were inoculated orally and peritoneally with macerates of ticks and fleas removed from 18 dogs symptomatic for visceral leishmaniasis (nine for ticks and nine for fleas). Six months later, the hamsters were sacrified and necropsied. Slide smears of spleen and liver, as well as PCR of these viscerae and IFAT (Indirect Fluorescent Antibody Test) from serum of hamsters were used as test to determine the parasite infection. Twenty hamsters inoculated by macerates of ticks gave positives results for *L. (L.) chagasi*. Sixteen hamsters that have been inoculated by macerates of fleas showed positive results. Eleven (68,7%) of them have been infected peritoneally and five (31,2%) orally. Tick macerates could infect 20 hamsters, being 14(70,0%) peritoneally and six (30,0%) orally.

Based on these findings, we suggest that the vectorial capacity of *R. sanguineus* and *C. felis felis* for *L. (L.) chagasi* should be evaluated further, opening new perspectives in the epidemiology of ZVL.

Apoio Financeiro: CAPES, CNPq.

VE52 - LUMINAL PH, PROTEOLYTIC ACTIVITY AND MACROSCOPIC ANATOMY OF THE DIGESTIVE TUBE OF *LUTZOMYIA LONGIPALPIS*' LARVAE (DIPTERA, PSYCHODIDAE).

do Vale, V.F; de Andrade, W.A.C; Delucca, H.M; Gontijo, N.F

Departamento de Parasitologia, ICB – Universidade Federal de Minas Gerais – UFMG. Laboratório de Fisiologia de Insetos Hematófagos

Phlebotomine sand flies are important in public health as vectors of *Leishmania* ssp. Despite their parasitological importance, the biological and molecular knowledge about immature forms is very restrict.

The objective of this work was to describe the macroscopic anatomy of the digestive tube of *Lutzomyia longipalpis* larvae as well as to measure the pH and characterize the proteolytic activity in their gut lumen. *Lutzomyia longipalpis* larvae have a bulk midgut flanked by a short and narrow foregut, and a hindgut with a conspicuous conical rectum.

pH measurements in the gut lumen were performed by using three different vital indicator dies mixed with larval meal. It was observed a pH gradient inside the midgut varying from >9 in the initial portion of this region to 6.5 just before the pylorus. A high pH like that observed in the initial portion is a common feature in detritivorous insects. Proteolytic activity was assayed by using the unspecific substrate azocasein and the synthetic substrates BApNA and N-CBZ-L-Phenylalanine-p-Nitroaniline which are specific to trypsin and chymotrypsin-like enzymes, respectively. Preliminary results, obtained with midgut extracts and azocasein as substrate, showed a remarkable proteolytic activity at higher pH values with one peak around pH 10.5. At least two peaks of trypsin activity were observed when BApNA was the substrate. The first peak had maximal activity at pH 8.5 and the other at pH 10. Just one peak at a high alkaline pH (pH 10 - 11) was observed when chymotrypsin was assayed.

More detailed studies concerning the proteolytic activity in the *Lutzomyia longipalpis*' larvae will be carried out.

Supported by: CNPq and FAPEMIG

VE53 - IDENTIFICATION AND CHARACTERIZATION OF GENES POTENTIALLY INVOLVED IN THE IMMUNE RESPONSE OF *LUTZOMYIA LONGIPALPIS*.

Christiane Marques, Adriana P.O. Araújo, J. Marcelo Ramalho-Ortigão, Yara M. Traub-Cseko

Department of Biochemistry and Molecular Biology, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil. marquesc@ioc.fiocruz.br

Lutzomyia longipalpis is the main vector of visceral leishmaniasis in Brazil. While parasite-insect vector interaction has been well studied in malaria, with the development of transgenic mosquitoes incapable of transmitting the parasite, little is know about the interaction leishmania-sand fly. We are studying molecules potentially involved in feeding and infection by leishmania in *Lutzomyia longipalpis*. We have done EST sequencing and differential display studies using RNAs extracted at different times after blood-feeding and infection with leishmania. Through DDRT-PCR we have identified some genes potentially involved in insect immune response: MAP-kinase, TGF-β, Cactus. It is well known that insects use a variety of strategies to fight pathogens, from physical barriers to defense peptides, that were shown to be produced by sand flies in response to bacterial infection. In *Drosophila*, specific recognition appears to be achieved by membrane receptors of the Toll family, equivalent to IL-1 receptor in vertebrates. Cactus is active in this response cascade, and a similar mechanisms could exist in sand flies. MAP-kinase, which is also involved in innate immune response, is very conserved among organisms as far as mammals, plants and arthropods. The gene found in *L.*

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

longipalpis showed similarity to the *Drosophila* gene. TGF-b may be involved in immune response, and is also very conserved. In *Anopheles stephensi*, TGF-b may have a function in the immune response against *Plasmodium* infection. The *L. longipalpis* TGF-b gene was identified through DDRT-PCR from RNA of sand flies fed on infected blood, indicating a potential immune response role for this molecule in this vector as well. We have previously partially sequenced *L. longipalpis* Map-kinase, TGF-b and Cactus genes. We are presently sequencing the whole gene by different techniques: probing cDNA libraries with the gene fragments in search for full sequences, 5'RACE and by the amplification of the 5' end of the gene by PCR amplification of a cDNA library, using a reverse homologous primer and a forward primer situated on the cloning vector. Fragments have been obtained and are presently being sequenced.

Supported by PAPESIII-Fiocruz, PDTIS, CNPq.

VE54 - CHARACTERIZATION OF TRYPSIN GENES IN *LUTZOMIA LONGIPALPIS*

Barroso, R.M.; Carvalho, S.T.; Ortigão-Farias, J.R.; Traub-Cseko, Y.M.

Department of Biochemistry and Molecular Biology, Oswaldo Cruz Institute, Fiocruz, Av. Brasil 4365, Rio de Janeiro, 21045-900, Brazil. sebatc@bol.com.br

According to WHO, leishmaniasis is among the main infectious disease and is widely distributed around the Americas. In Brazil, the number of cases is increasing gradually over the last 20 years. *Leishmania* is transmitted to mammal hosts from wild reservoirs by Phlebotomine sand flies. *Lutzomyia longipalpis* is the main vector of *Leishmania (L.) chagasi,* that causes visceral disease in Brazil. We are studying molecular aspects of blood feeding and interaction between *Leishmania* parasites and their insect vector host. Knowledge of the vector biology and physiology can be an important tool to intervene in this interaction mechanisms. We intend to characterize specific molecules that might have a significant role in feeding and in this interaction and use such molecules as potential targets for the development of new strategies in the fight against the spread of leishmaniasis. There is already lots of information from studies with the malaria vector *Anopheles gambiae.* Various mosquito genes which may participate in feeding and in parasite interaction have been identified and characterized. Much of the attention has been placed on midgut specific genes and promoter elements. Midgut-specific proteins like trypsin, chymotrypsin, chitinase and others are being considered potential targets for the development of transgenic insect populations unable to harbor a parasite or transmit it to the vertebrate host. Although most of the data available comes from mosquitoes, this same approach can be applied to other insects such as phlebotomine sand flies. We isolated, from an expression library from blood-fed midgut, trypsin codifying cDNAs. These genes are regulated by blood ingestion and are probably involved in blood digestion. Partial sequencing of these clones showed that there are at least two different genes, with similarity to other insect trypsins. We are presently completing the sequencing by either 5'RACE or by PCR from the expression library, using internal reverse primers and a forward primer situated on the plasmid. We are also interested in characterizing a genomic clone, for the identification of introns and regulatory sequences. For that a genomic library in EMBL3 is being probed with the trypsin gene fragments. Positive clones have been obtained. These clones are being characterized by mapping, hybridization and sequencing.

Supported by PAPESIII-Fiocruz, PDTIS, Faperj, CNPq.

VE55 - AMERICAN TEGUMENTAR LEISHMANIASIS IN THE PONTAL OF PARANAPANEMA RIVER, SP, BRAZIL, AND THE RELATIONSHIP WITH MST (LANDLESS MOVEMENT FOR LAND).

[1,2]Alessi,C.A.C.; [3] Pereira,A.P.; [2] Alves,J.R.; [4] Corbett,C.E.P.

[1]Making Master degree in FMUSP (Medical school of São Paulo University), [2] Department of Parasitology of Unoeste University; [3]Laboratory of Pathological Anatomy and Cytopathology of Pres. Prudente; [4] Laboratory of Pathology of Infectious Diseases of FMUSP.

Introduction: American tegumentar leishmaniasis (ATL) is a zoonosis and transmission depends on many factors due to ecological changes or by invasion of man to natural niches where vectors and reservoirs are present. Seasonal variations and different susceptibility of the population also happens.

Objective: To present a survey of the American tegumentar leishmaniasis (ATL) in Pontal of the Paranapanema River, SP.

Methods: Retrospective and prospective study of cases of leishmaniasis in the Pontal of the Paranapanema River was carried out. The clinical forms were obtained from data of the Center of Epidemiological Vigilance (CEV), of the Health State Secretary of São Paulo, from May 1995 to December 2001. The studied variables were submitted to statistical analysis.

Results: The total of registered cases were 89. Most notified cases were from the district of Teodoro Sampaio (29,2%). The predominant clinical form was cutaneous lesion (78,65%). The most frequent lesion was in exposed areas of the body, mostly in the down limbs (36,36%). The higher occurrence was in male (67,42%). Individuals with age up to 54 years corresponded to 75% of the cases. The most notified cases were from rural settlements or MST (Landless Movement for Land) camps existing in the Pontal of Paranapanema. The higher number of notifications was in the winter and spring.

Conclusion: The data show the transmission in Pontal of Paranapanema occurs when man gets into the natural habitat of the zoonosis. Based in the epidemiological data, ATL occurs predominantly in the male, with age from 30 to 60 years, in the classical cutaneous form, where most with rural occupation or habitation.

VE56 - CAPTURED *LUTZOMYIA INTERMEDIA* SAND FLIES ARE SUCCESSFUL INFECTED WITH *LEISHMANIA BRAZILIENSIS* USING AN EXPERIMENTAL MODEL.

Souza, APA*;Bahia-Nascimento, AC*; Secundino, NFC**; Prates, D*; Barral-Netto, M*; Barral, A*; Miranda, JC*; Pimenta, PF**.

*Centro de Pesquisas Gonçalo Moniz- FIOCRUZ/BA. ** Centro de Pesquisas René Rachou- FIOCRUZ/MG.

Lutzomyia intermedia has been demonstrated as major vector of the ACL due to *Leishmania (V.) braziliensis* in Southeast Brazil. The aim of this study is to understand interactions between *Leishmania (V.) braziliensis* and its vector *Lutzomyia intermedia*, both from an endemic area of American Cutaneous Leishmaniasis (ACL). About two-thousand *L. intermedia* sand flies were captured and subjected to infection with *L. (V.) braziliensis* amastigotes. The flies were allowed to feed throughout a chick skin membrane in an artificial feeding device containing heparinized mouse blood seeded with parasites for 3h. After that, flies were maintained in sugar diet. Following infection, the gut of each sand fly was dissected in differents time points until to complete the digestion (1st to 10th day). The guts were examined to observe the presence, location, morphology and density of the parasites. Three-hundred and eighty-eight sand flies were examined and we observed an infection load of 86.5% at the first day and 42.9% at the last day after the blood meal. Interestingly, we demonstrated that procyclic promastigotes represented 100% of the population at the 1st and 2nd days, and metacyclic forms accounted for 40% at day 5 following infection. Haptomonads, nectomonads and paramastigotes promastigotes were present at different levels during the study.

This work is basis for subsequent studies which are being developed in our laboratory for better understanding interactions between *L. (V.) braziliensis-L. intermedia*, including comparison with others vectors present in the same endemic area, such as *Lutzomyia whitmani* and *Leishmania (L.) amazonensis.*

Support: CNPq, FIOCRUZ, FAPEMIG, FAPESB.

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

VE57 - SPATIAL ORGANIZATION AND STRUCTURAL MODIFICATION OF MIDGUT MUSCLE NETWORK RELATED WITH BLOOD MEAL IN *LUTZOMYIA LONGIPALPIS* AND *PHLEBOTOMUS DUBOSCQI* SANDFLIES, VECTORS OF LEISHMANIASIS.

[1] Secundino NFC, [2] Hajamova M, [2] Wolf P and [1]* Pimenta PFP

[1]Laboratory of Medical Entomology, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Fiocruz-MG, Brazil and [2]Department of Parasitology, Charles University, Prague, Czech Republic. pimenta@cpqrr.fiocruz.br

Sand flies are vectors of leishmaniasis in vertebrates including man. Sand flies are able to feed on plant nectars and vertebrate blood, but hematophagy is an essential and exclusive behavior of females, which needs the blood meal to develop its eggs. The alimentary tract of sandflies is divided into three regions: foregut, midgut and hindgut. The midgut itself can be divided into two topographic regions according to their location: thoracic and abdominal midguts. The blood meal is stored and digested in the midgut. Muscle fibers are responsible for extensive changes in the midgut volume during the blood ingestion and digestion. Therefore, the knowledge of the structure of the muscle fibers present in the midguts of vectors of human diseases is important in order to correlate it with the organ functions. We describe and compare the spatial organization and the modifications of the midgut muscle fibers related with blood meal process. The midguts were morphological analyzed immediately after blood meal ingestion until its complete digestion, following the periods of 0 to 72 hours for *Lu. longipalpis* and 0 to 96 hours for *P. dubosqi*, since they finished their digestive processes at distinct times. The muscle components are placed over the entire midgut region as circular and longitudinal fibers forming a well-arranged muscle network. The muscle fibers are striated due to alignment of the Actin filaments clearly demonstrated by Phalloidin labeling and scanning electron microscopy. The thoracic midguts of the two sand flies do not distend after blood meal and do not change the arrangement of their muscle networks. On the opposite, their abdominal midguts suffer several modifications in the muscle network organization, which appeared to be completely related to the blood meal journey into the midgut. It is also remarkable to observe that after the digestion of the blood meal was finished, the muscle fibers of the *P. duboscqi* midgut returned to a better-organized muscle network than the *L. longipalpis* midgut fibers. This fact could be due to the differences in the arrangements of the muscle network of the two sand fly midguts, or even because of the distinct biochemical compositions of the midgut muscles of the two sand flies species, which were not detected by ours observations. In conclusion, the actin labeling with fluorescent Phaloidin and SEM allowed us to visualize in details, for the first time, the muscle organization of the midguts of two sand fly species, important vectors of leishmaniasis. This muscle network presented structural modifications related with the ingestion, storage and digestion of the blood meals in the midguts. This knowledge is important for a better understanding of the organ physiology and disease transmission by these insects.

This knowledge is important for a better understanding of the organ physiology and disease transmission by these insect vectors.

It is also remarkable to observe that after the digestion of the blood meal was finished, the muscle fibers of the *P. duboscqi* midgut returned to a better-organized muscle network than the *L. longipalpis* midgut fibers..

Financial support: CNPq, Fapemig and Fiocruz.

VE58 - STUDY OF THE OVARIAN DEVELOPMENT AND EGG EXOCHORION OF *CULEX QUINQUEFASCIATUS* USING SCANNING ELECTRON MICROSCOPY

Nacif-Pimenta R., Secundino, N. F. C. and Pimenta P. F. P.

Laboratory of Medical Entomology, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Fiocruz-MG, Brazil. E-mail: pimenta@cpqrr.fiocruz.br and rafanp@cpqrr.fiocruz.br

Culex quinquefasciatus is the mosquito vector of *Wuchereria bancrofti*, parasite that causes human filariasis in Brazil. It is considered a tropical–cosmopolitan with a high level of anthrophylia. The feeding adaptation allows it to feed during nighttime, which coincides with the peripherical circulation of the parasite optimizing vector competence. In order for the hematophagus female to lay healthy viable eggs, the ingestion of blood from a warm-blooded vertebrate is needed to complete the protein requirement for the egg's development.

The female reproductive system consists of spermtheca, accessory glands and ovaries, which are composed of ovarioles that will develop into eggs. This study focuses on different stages of the ovarian maturation and development, and the hatched eggshell using the scanning electron microscope (SEM). The mosquitoes were blood fed on quails (*Coturnix* sp.). Ovaries of five females were dissected at different times after blood meals (from 0-72h). Samples were processed and observed in the SEM. The measurements were made using microscope software. The size and the morphology of ovaries from 0 to 6h were similar (357µm X 140µm). These ovaries have dense and compact membranes, which appear to be composed of overlapping filaments of different sizes. At 6h, these membranes were ruptured and it was possible to observe the ovarioles measuring 15um X 20um. At 12h, a similar ruptured membrane allowed us to see intact and ruptured ovaries measuring 523µm X 180µm and 493µm X 203µm, respectively. The ovaries of 24h, 36h and 48h with ovarian membrane measured 560µm X 262µm and without the membrane measured 764µm X 430µm. The ovarioles from these times, which were seen under the ruptured membrane, measured 72um X 71um. At 72, the ovaries (1,580µm X 720 µm) and ovarioles (439µm X 101 µm) increased in size, and the ovarian membrane was very thin. The ovarioles were almost completely formed and lost another membrane that exposes their adherent surface of the egg. In the hatched eggshells, it was possible to see the exochorion, which is the outer layer of the egg. The egg's exochorion was composed of tubercles arranged in orthogonal arrays. The operculum from where the larvae will escape was also seen. In conclusion, it appears that in the first hours, the ovaries do not show any significant changes but around 36h there is a rapid growth, probably due to the end of the digestion process. The ovarian membrane is responsible for containing the ovarioles in formation. In this study was possible to observe several ultrastructural events related with the mosquito egg´s development.

VE59 - MOLECULAR ANALYSIS OF GLUTATHIONE PEROXIDASE GENES: COMMON CHARACTERISTICS SHARED BY PLANTS, ARTHROPODS AND YEAST

Marta Citelli dos Reis and Pedro Lagerblad Oliveira

Departamento de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil

Glutathione peroxidase (GPX) is one of the most important enzymes involved in cell redox regulation. GPx is usually described as a selenoenzyme. But, until now, all glutathione peroxidases homologous described in arthropods and yeast do not have the amino acid selenocysteine and plants have just one example of GPX-Se[1]. We have recently cloned a GPX gene homologous in the cattle tick *Boophilus microplus* (Bmgpx) which shares about 58% and 54% amino acid sequence identity with plant (*Momordica charantia*) and mammalian (*Mus musculus*) PHGPXs, respectively. As other arthropods GPX homologous, Bmgpx do not code for a selenoenzyme, since it does not have the opal codon UGA. Molecular analysis of GPX genes from arthropods, yeast and plants revealed that they have two common features: they don't code for selenoenzymes and they are similar to phospholipid hydroperoxide glutathione peroxidase (PHGPX).